Particulate organic matter: Vector for carbon export, indicator for aquaculture impact and microbial hotspot

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SUMMARY

A large proportion of global carbon lies within the ocean. Here, the particulate fraction (compounds >0.45 µm) can serve as vectors for horizontal and vertical carbon export, indicators for aquaculture impact and microbial habitats, hotspots and refuges.

In Eastern Boundary Currents, such as the Canary Current system off NW Africa, upwelling of nutrient-rich waters stimulates primary productivity. This results in substantial horizontal and vertical export of particulate organic matter (POM). Meanwhile, heterotrophic bacteria are involved in the degradation of the organic matter. On the one hand this can result in a less efficient carbon sequestration but on the other hand this may stimulate food webs in the surface waters. Thereby, the bacterial community composition (BCC) is a major factor, influencing organic carbon (from here on carbon) turnover capacities. Especially due to global climate change, the vertical carbon export, termed "biological carbon pump" (BCP), is of great interest. However, despite the important role of bacteria in determining the efficiency of the BCP, little is known about the BCC off NW Africa. Therefore, the first objective of this thesis is the description of the free-living (FL) and particle-attached (PA) BCC off NW Africa and the identification of potential key players in carbon degradation. Results reveal high relative abundances of particle-degrading bacterial groups, suggesting a key role of these bacteria in the turnover of carbon. Furthermore, simulated sinking of POM resulted in a shift in the attached BCC, which was not observed in simulated horizontal POM export. As a comparable shift has previously been shown to lead to reduced respiration rates, future research might reveal another possible microbial mechanism involved in increasing vertical carbon export efficiencies.

Coastal aquaculture practices in the tropics are often accompanied by eutrophication and high POM loads in their effluents. Here, the horizontal export of high amounts of POM can have detrimental effects on adjacent ecosystems (e.g. seagrass beds and coral reefs) and their functioning. The vertical export of POM, especially in shallow coastal regions, often causes the accumulation of thick layers of organic matter on the sea floor. In both cases, bacterial carbon degradation is stimulated by high POM concentrations, leading to a substantial drawdown of O_2, at times even hypoxia. In Bolinao, Philippines, recurring hypoxic events in the water column lead to major fish kills. Nevertheless, so far POM-attached microbial respiration rates have not been quantified, yet. Therefore, another aim of this study was the quantification of PA microbial respiration rates to assess if their activity can contribute to

recurring water column hypoxia in Bolinao. Results reveal high carbon specific respiration rates, indicating that PA microbial carbon degradation has the potential to contribute to water column hypoxia. Furthermore, due to direct impact of aquaculture practices on the POM (e.g. due to feeding), POM characteristics, such as carbon to nitrogen (C:N) ratio, stable isotope values, POM concentrations, sinking velocities, bacterial alpha diversity and their BCC, may reflect the aquaculture impact. Therefore, we tested the possibility if POM characteristics can serve as indicators for aquaculture impact on coastal ecosystems. Results indicate a strong signal of aquaculture activities of POM characteristics, rendering them ideal indicators for aquaculture impact.

Despite the major ecological differences between the two study sites of this thesis, POM always served as a substrate for PA bacteria. However, microbial particle dynamics are very complex. Motility and chemotaxis allow certain bacterial groups to find and attach to substrates (POM). Some bacteria only loosely associate and detach again, if they encounter other hotspots in their vicinity. However, so far it is unknown how differences in substrate availability in the natural environment affect this process. Therefore, we aimed at determining the similarities of FL and PA bacteria in waters with high (deep-chlorophyll maximum) vs. low (meso- to ultraoligotrophic waters) substrate availabilities. Results indicate that FL and PA bacteria are more similar at the DCM. This may indicate that substrate availability stimulates the exchange of PA bacteria between substrates, because to reach the next hotspot, they have to cross the FL fraction. Additionally, also vertical and horizontal export may result in exchange of PA bacteria, because the substrate-attached bacteria are subjected to different water masses and hence surrounding BCC. Therefore, we examined if the BCC of substrate-attached bacteria is also in exchange with surrounding BCC during horizontal and vertical export. Although we observed that the majority of BCC was determined at the beginning, we identified changes in substrate-attached bacteria when subjected to different water masses, which indicates exchange (e.g. due to scavenging) with the surrounding sea water BCC.

Overall, the results presented in this thesis advance our understanding regarding the microbial component of the BCP in Eastern Boundary Currents, about biogeochemical processes on aquaculture-derived POM and about microbial particle dynamics. Furthermore, they indicate how processes occurring on the microscale are relevant for current global challenges, such as climate change and the degradation of coastal ecosystems.

ZUSAMMENFASSUNG

Ein großer Anteil des globalen Kohlenstoffs ist in den Ozeanen gespeichert. Hierbei kann der partikuläre Anteil (>0.45 µm) des marinen Kohlenstoffs als Vektor für horizontalen und vertikalen Kohlenstoffexport, als Indikator für die negativen Auswirkungen von Aquakulturpraktiken, sowie als mikrobielles/ r Habitat, Hotspot und Refugium dienen.

In östlichen Randströmungen, wie zB. dem Kanarenstrom vor der Küste NW Afrikas, stimuliert der Auftrieb von nährstoffreichem Tiefenwasser die Primärproduktion. Dies führt zu einem erheblichen horizontalen und vertikalen Export von parikulärem organischen Material (POM). Unterdessen sind heterotrophe Bakterien involviert in dem Abbau des organischen Materials. Dies kann einerseits zu einer weniger effizienten Kohlenstoffsequestrierung führen, aber andererseits das Nahrungsnetz im Oberflächenwasser antreiben. Hierbei ist die Zusammensetzung der bakteriellen Gemeinschaft (BG) ein entscheidende Faktor, der die Leistungsfähigkeit des Kohlenstoffabbaus beeinflusst. Vor allem durch den globalen Klimawandel ist das Interesse an dem vertikalen Kohlenstoffexport, auch bekannt/ bezeichnet als „Biologische Kohlenstoffpumpe“ (BKP), groß. Obwohl, Bakterien die Effektivität des Kohlenstoffexports beeinflussen können, ist die BG vor NW Afrika wenig erforscht. Deshalb war das erste Ziel dieser Arbeit die Beschreibung der frei-lebenden (FL) und partikel-assoziierten (PA) BG vor NW Afrika, sowie die Identifikation von potenziellen Schlüsselbakterien im Kohlenstoffabbau. Die Ergebnisse offenbaren hohe relative Abundanzen von Bakterien, die für den Abbau von POM bekannt sind. Dies deutet darauf hin, dass diese Bakterien eine Schlüsselrolle im Kohlenstoffabbau vor NW Afrika einnehmen. Zusätzlich hat die experimentelle Simulation von sinkendem POM zu einer großen Verschiebung in der substrat-assoziierten BG geführt. Diese Verschiebung konnte bei der Simulation von horizontalen POM Export nicht beobachtet werden. Da bereits gezeigt wurde, dass eine vergleichbare Verschiebung in der BG zu geringeren Respirationsraten führte, könnten zukünftige Untersuchungen möglicherweise einen weiteren mikrobiellen Mechanismus aufdecken, der zu einer höheren Effizienz des vertikalen Kohlenstoffexports führt.

Küstennahe Aquakulturpraktiken in den Tropen sind oft begleitet von Eutrophierung und hohen POM Konzentrationen. In diesen Systemen kann der horizontale Export von hohen Mengen an POM schädliche Auswirkungen auf benachbarte Ökosysteme (zB. Seegraswiesen oder Korallenriffe) und deren Funktionen haben. In flachen Küstenregionen kann der

vertikale POM Export zur Anhäufung von dicken Schichten organischen Materials auf dem Meeresboden führen. In beiden Fällen wird der bakterielle Kohlenstoffabbau durch hohe POM Konzentrationen stimuliert, welches zu einer erheblichen Sauerstoffabsenkung, bis hin zu Hypoxie, führen kann. In Bolinao, Phillippinen, hat wiederkehrende Hypoxie in der Wassersäule bereits zu großem Fischsterben geführt. Trotzdem wurden bis jetzt die Respirationsraten von POM-assoziierten Bakterien noch nicht quantifiziert. Deshalb war ein weiteres Ziel dieser Arbeit die Quantifizierung der Respirationsraten von POM-assoziierten Mikroben. Die Ergebnisse offenbaren sehr hohe mikrobielle Respirationsraten, was darauf hindeutet, dass die Bakterienaktivität das Potenzial hat, zu geringen Sauerstoffkonzentrationen in der Wassersäule sowie der wiederkehrenden Hypoxie beizutragen. Durch den direkten Einfluss von Aquakulturpraktiken auf das POM (zB. Füttern der Fische) kann es sein, dass POM Charakteristika, wie zum Beispiel der Kohlenstoff/ Stickstoff Anteil, stabile Isotope, POM Konzentrationen, Sinkgeschwindigkeiten, bakterielle Alpha-Diversität und BG, die Auswirkungen von Aquakulturen widerspiegeln. Aus diesem Grund haben wir getestet, ob POM Charakteristika als Indikatoren für Auswirkungen von Aquakulturpraktiken auf küstennahe Ökosysteme dienen können. Die Ergebnisse zeigen, dass POM ein starkes Signal der Aquakulturaktivitäten speichert, sodass seine Charakteristika als Indikatoren für die Auswirkungen von Aquakulturpraktiken geeignet sind.

Trotz der großen ökologischen Unterschiede zwischen den beiden Untersuchungsgebieten, hat POM immer als Substrat für PA Bakterien gedient. Allerdings sind die mikrobiellen Dynamiken an Partikeln sind sehr komplex. Beweglichkeit und Chemotaxis erlauben es bestimmten Bakteriengruppen POM zu finden und sich daran zu binden. Manche dieser Bakterien binden sich aber nur lose an das Substrat, sodass sie sich jederzeit wieder lösen können, wenn sich ein anderer Hotspot in der Nähe befindet. Bis jetzt war es unklar, wie Unterschiede in der Substratverfügbarkeit in der natürlichen Umgebung diese Prozesse beeinflussen. Aus diesem Grund haben wir die Ähnlichkeit von FL und PA Bakterien in Gewässern mit hoher (zB. im tiefen Chlorophyllmaximum) und niedriger (in mesotrophen bis ultraoligorophen) Substratverfügbarkeit untersucht. Die Ergebnisse zeigen, dass FL und PA Bakterien bei tiefen Chlorophyllmaxima sich am ähnlichsten sind. Dies könnte bedeuten, dass Substratverfügbarkeit den Austausch zwischen PA Bakterien antreibt, da sie, um den nächsten Hotspot zu erreichen, die FL Fraktion überqueren müssen. Auch der horizontale und vertikale Export von Partikeln könnte zu Veränderungen in der PA BG führen, da diese während des Transports unterschiedlichen Wassermassen und damit BG ausgestzt sind. Daher haben wir untersucht, ob sich die PA BG während horizontalem und vertikalem POM Export verändert.

Zwar haben wir beobachtet, dass der Großteil der BG am Anfang festgelegt wird, aber trotzdem kleine Veränderungen in der BG zu beobachten sind, wenn sie anderen Wassermassen ausgesetzt werden. Auch diese Ergebnisse deuteen auf den Austausch von PA Bakterien (zB. durch Kollision mit kleineren Partikeln) hin.

Insgesamt tragen die Ergebnisse aus dieser Arbeit zu einem besseren Verständnis über den mikrobiellen Anteil der BKP in östlichen Randströmungen, über biogeochemische Prozesse von POM aus Aquakulturen und über mikrobielle Partikeldynamiken bei. Außerdem demonstrieren die Ergebnisse, wie wichtig mikroskalige Prozesse für globale Herausforderungen, wie den Klimawandel oder die Degradierung von küstennahen Ökosystemen, sein können.

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CONTENTS

SUMMARY III
ZUSAMMENFASSUNG V
ACKNOWLEDGEMENTS VIII
CHAPTER I 1
INTRODUCTION 2
Organic matter in the marine environment 2
Marine aggregates 4
Organic particles are integral parts of marine ecosystems 5
Aims of this thesis 11
Publication overview 13
REFERENCES 15
CHAPTER II 19
ABSTRACT 20
INTRODUCTION 20
MATERIALS AND METHODS 22
RESULTS 24
DISCUSSION 27
CONCLUSION 29
REFERENCES 30
CHAPTER III 33
ABSTRACT 35
INTRODUCTION 36
MATERIALS AND METHODS 40
Chemical sea water parameters 45
Video determination of aggregate sizes, sinking velocities and aggregation volume 46
Flow chamber measurements to determine the size, sinking velocities and O_2 fluxes of selected aggregates 46
Organic carbon content of aggregates 47
Scanning electron microscopy (SEM) imaging 47
FL, PA and AG microbial community composition 48
Bioinformatic analyses 48
Statistical analyses 49
RESULTS 50
Sea water parameters 50
Aggregation volume 52

Composition of aggregates 54
Sinking velocities of aggregates 54
Carbon specific respiration rates of aggregates 55
Microbial community composition 57
DISCUSSION 60
Highest aggregation volume and sinking velocities in E-upw 60
Aggregate- and particle-attached microbial degradation of POM 61
Flow through rolling tanks: Improvements for future studies 61
Bacterial attachment to precolonized aggregates 62
Shifts in BCC on marine snow during sinking: Could it affect the efficiency of the BCP? 63
Differences in BCC depending on particle size 63
Single aggregate heterogeneity 64
CONCLUSION 65
REFERENCES 66
Acknowledgements 75
Funding 75
SUPPLEMENTARY MATERIAL 76
CHAPTER IV 81
ABSTRACT 83
INTRODUCTION 84
MATERIALS AND METHODS 87
Sampling site description 86
Environmental water parameters 88
GoPro measurements of particle abundances and their size distributions 89
Sediment trap application 90
CN content of particles 91
Stable isotope signature in particulate matter 91
Microscopic analysis of aggregates 92
Determination of bacterial communities on S and L particles and on single aggregates 92
Flow chamber: Equivalent spherical diameters and sinking velocities 93
Microelectrode respiration measurements 94
Statistical analysis 94
Future potential of this data set 95
RESULTS 96
Environmental parameters 96
GoPro as functional particulate matter detection system 98

Particle abundances determined by GoPro measurements 98
CN content of particles 99
Stable isotope signature of particles 101
Bacterial alpha diversity 102
Bacterial beta diversity 103
Bacterial community composition of S and L particles 103
The four major types of aggregates 106
Flow chamber analysis and microelectrode respiration measurements 107
BCC and oxygen consumption on single aggregates 108
DISCUSSION 110
Establishment of GoPros as functional particle detection system 110
Tidal effects on environmental parameters 111
Particle abundances and biogeochemistry vary with tide and over time 111
Aquaculture impacts on bacterial alpha diversities and community composition 113
CONCLUSION 115
REFERENCES 116
Funding 122
Acknowledgements 122
Conflict of interest 122
SUPPLEMENTARY MATERIAL 123
CHAPTER V 139
GENERAL DISCUSSION 140
Particulate organic matter as vector for horizontal and vertical carbon export in EBCs 140
Ecosystem responses to eutrophication 144
Organic particles: micro-habitats for marine bacteria 147
CONCLUSION AND OUTLOOK 149
REFERENCES 152
CHAPTER VI 161
ABSTRACT 162

CHAPTER I

INTRODUCTION

Around 71% of surface of our planet is covered by oceans. These major ecosystems do not only play an important role in the regulation of global climate, they also support the livelihoods and food security of a large part of our population. In fact many low-income citizens largely rely on the ocean for animal protein supply (Kent 1997).

Organic matter in the marine environment

Nowadays, the largest part of the actively cycled carbon lies within the oceans (Post et al. 1998). Traditionally, the marine organic carbon (C) pool has been divided into the dissolved organic matter (DOM) and the particulate organic matter (POM; Figure 1). According to this definition, organic compounds < 0.45 µm are defined as DOM and those exceeding 0.45 µm belong to the POM (Azam and Malfatti 2007).

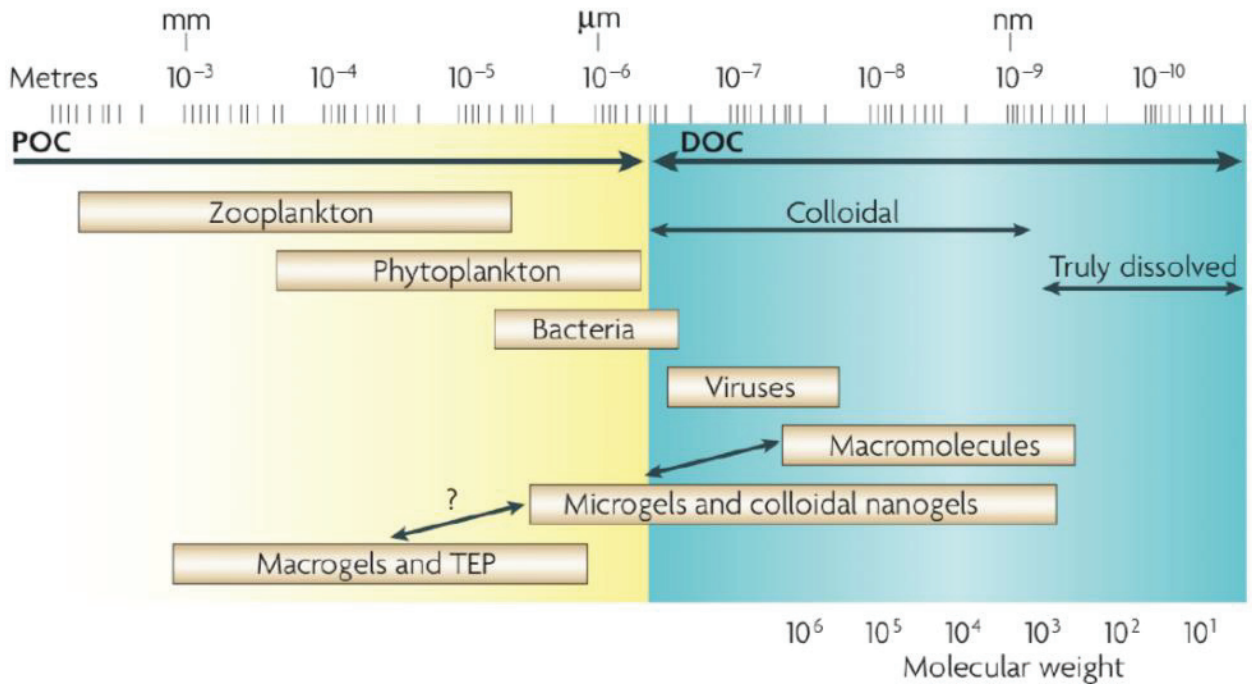

Figure 1. Size continuum of the marine carbon pool (Source: Azam & Malfatti (2007))

While DOM includes e.g. polysaccharides, lipids and proteins, POM consists of living organisms, such as phytoplankton cells, and dead material, including detritus, faecal pellets and other waste products (Menzel and Ryther 1964). POM is formed through various activities but it is generally a mixture of both biotic (e.g. primary productivity and grazing) and abiotic (e.g. agglomeration and flocculation) processes (Fowler and Knauer 1986).

Although the particulate organic fraction only makes up ca. 1 % of the marine carbon pool, POM plays an important role in the regulation of our global climate (Benner et al., 1997).

Besides the particulate and dissolved fraction the hydrogels have received increasing attention. Marine gels, which span the whole size range from dissolved to particulate (100 µm or larger) organic material, are ubiquitous in the marine environment (Verdugo et al. 2004).

One of the best studied groups of hydrogels are mucopolysaccharide gels, such as the transparent exopolymer particles (TEP; Passow 2002; Verdugo et al. 2004). TEP (Figure 2) are abundant types of macrogels, which are formed abiotically from precursors (acidic polysaccharides), released among others by phytoplankton (Passow 2002).

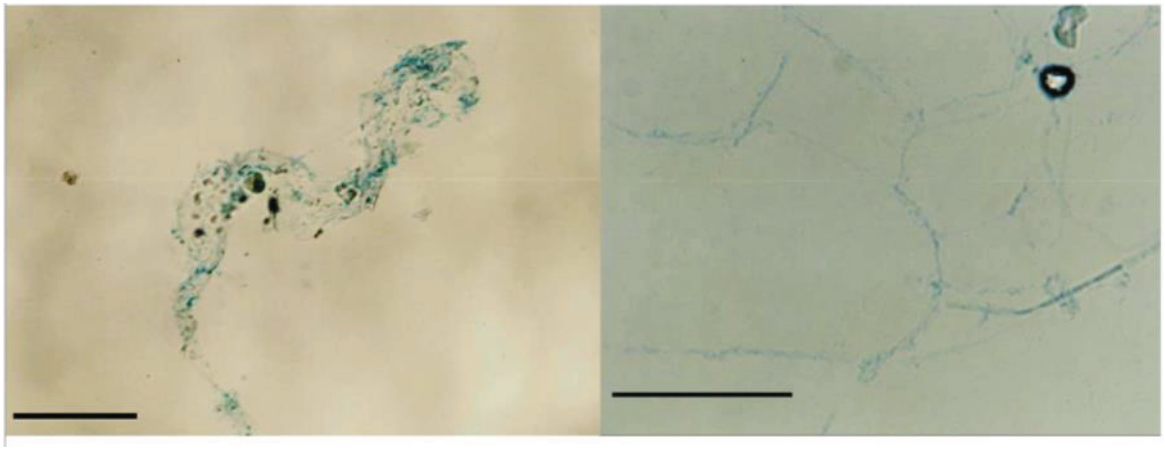

Figure 2. Transparent exopolymer particles stained with alcian blue (scale bar = 0.1mm; Source: Passow, 2002)

TEP form the matrices of marine aggregates and are thus thought to play an important role in marine biogeochemical cycles (Passow 2002).

Marine aggregates

Marine aggregates (Figure 3) are made up of a variety of small particles (e.g. detritus, microbes and minerals) embedded in a gel-like matrix (Riley, 1963). They are formed depending on several factors: background particle concentration, their stickiness and physical parameters such as sinking velocity, turbulence and Bownian motion (e.g. Dash et al., 2015). If the aggregates exceed a size of 0.5 mm they are referred to as "marine snow" (Suzuki & Kato, 1953). Both macro- and micro-aggregates are abundant in the water column and have important implications for the functioning of the biological pump.

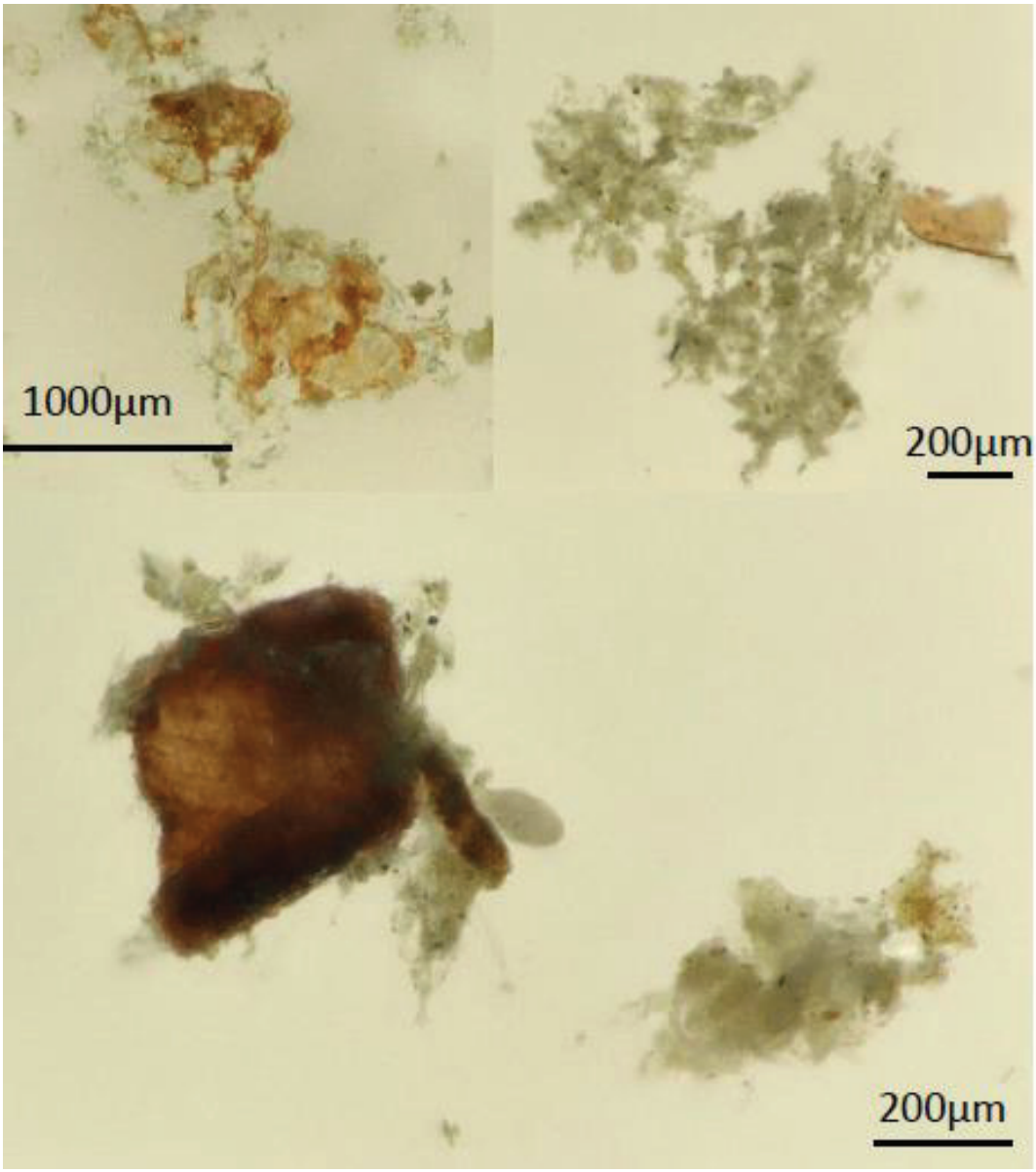

Figure 3. Marine aggregates are characterized by a gel like matrix, which glues different organic and inorganic particles together.

Organic particles are integral parts of marine ecosystems

While aggregates refer to an agglomeration of different materials, particles are individual entities, such as single phytoplankton cells. However, in the scientific literature, microbiologists often use the term "particle" as a shortcut for particulate (organic) matter, which includes both particles and aggregates. Unless indicated otherwise, the microbiologists' definition is adapted in this thesis. If the term "aggregate" is used, this means a particle was clearly identified as an aggregate (e.g. under the microscope).

In neritic as well as ocean regions, organic particles are integral parts of the ecosystems. In the oceanic regions, they are well known for their role in the BCP (De La Rocha and Passow 2007). In coastal regions, high amounts of POM may quickly reach the shallow sea floors, where an overload of organic particles can lead to adverse effects for benthic communities (Holmer and Kristensen 1992).

In this thesis, the role of organic particles in the BCP is elucidated first, before exploring the potential of organic particles as indicators for anthropogenic impact on the coastal environment.

The Biological carbon pump

Atmosphere and ocean surface constantly exchange gases to each equilibrium. Therefore, the oceans are important sinks for anthropogenic CO_2 emissions, of which they have already taken up around 1/3rd (Sabine et al. 2004). Via the abiotic and biological carbon pumps, the ocean has the capacity to store carbon for centuries or longer (Basu and Mackey 2018). Thus, understanding these two pumps, which function against a concentration gradient of carbon from the surface to the deep ocean, is very important for global carbon cycles.

The biological carbon pump (BCP, Figure 4) can be roughly divided into three parts: the production, sinking and remineralization of POM. Photosynthetically active organisms, including single celled phytoplankton, fix dissolved inorganic carbon into biomass, which is then exported to below the thermocline. Although the sinking velocities of single cells are very slow (Smayda 1969), their export to the deep ocean is facilitated by two possibilities: a) the microalgae may be ingested by larger organisms, such as zooplankton, which excrete the remainders as compact, fast sinking fecal pellets b) the dying phytoplankton cells, which may excrete sticky gels, such as TEP, collide with other particles and form large aggregates and

thereby increase their sinking velocities. The bio-remineralization of POM is driven by heterotrophic bacteria, which have the ability to degrade organic matter.

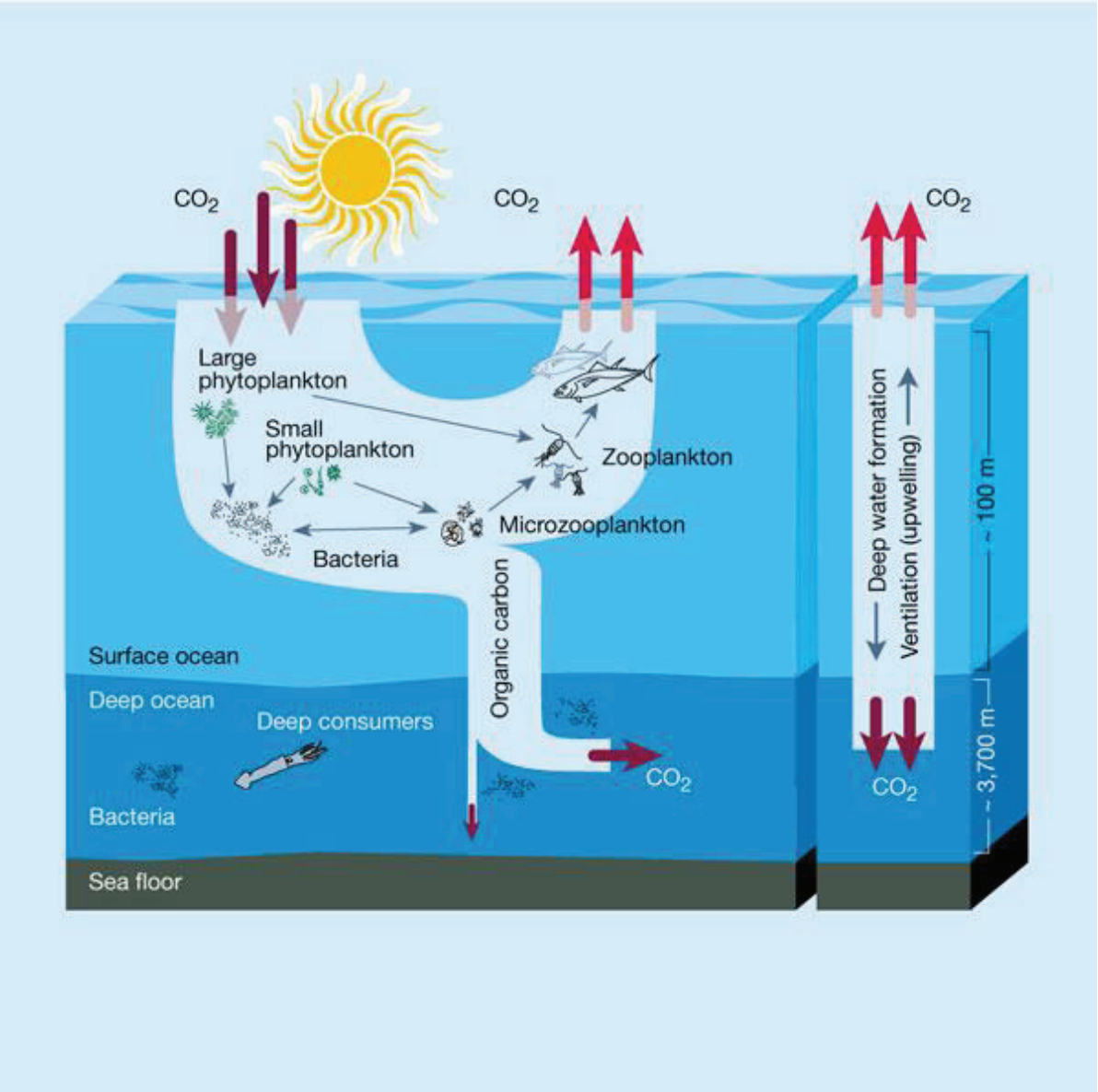

Figure 4. Two carbon pumps are involved in the export of carbon from the surface layer to the deep ocean: the physical (right) and the biological carbon pump (left). Source: (Chisholm 2000)

Degradation of organic material by heterotrophic bacteria

Marine bacteria can be categorized as particle-attached (PA), free-living (FL) or alternating between the two lifestyles (Grossart 2010). While PA bacteria are associated with organic particles and are characterized by a higher capacity to degrade polymeric substances (Lyons and Dobbs 2012), FL bacteria are suspended in the water and thrive off DOM.

In a process known as the "microbial loop" (Azam and Malfatti 2007), PA and FL heterotrophic bacteria hydrolyze POM and DOM and release bioavailable products into the surrounding water. This in turn fuels the food web from the bottom up by supporting the production of regenerated biomass.

In oligotrophic environments, many microorganisms, such as heterotrophic bacteria and protists, benefit from the colonization of particles, due to higher concentrations of organic and inorganic nutrients in particles compared to the surrounding seawater (Alldredge and Youngbluth, 1985; Simon, et al., 2002). Typically, PA bacteria possess respiration rates of around 0.1-0.2 d^{-1} (Ploug et al., 1999). Hence, the activity (OM degradation) of PA bacteria can influence the efficiency of the BCP by reducing the OM, which is exported to below the thermocline. Hereby, the bacterial community composition (BCC) has a major influence on the capacity for OM degradation (Enke et al. 2018).

Study Site I: The coastal and oceanic waters off Mauritania and Senegal

Off the coast of North West Africa, one of the major Eastern boundary currents (EBC), the Canary Current, transports cold waters southwards. EBCs, including the Canary Current, are among the most productive marine regions of the world (Carr 2002). Due to its capacity to sequester large amounts of organic carbon, this region serves as a major global carbon sink (Arístegui et al. 2009; Fischer et al. 2016). Off Mauritania and Senegal, seasonal upwelling is influenced by changes in trade winds and the migration of the Intertropical Convergence Zone towards the relatively warmer hemisphere between 2°N during Boreal winter and 9°N during summer (Schneider et al. 2014). In 2016, the METEOR 129 (M129) cruise led to the Gulf d'Arguin (Northern Mauretania) and Sine Saloum (Senegal) to provide one of the first detailed molecular studies on the FL and PA communities and their influence on the BCP in EBCs.

Eutrophication of coastal environments

Since the industrial revolution, anthropogenic input of nutrients and organic matter into the coastal environments has increased tremendously (Smith et al., 1999). Excess nutrients and OM in an environment may lead to eutrophication (Figure 5), which can affect biogeochemical cycles and have detrimental consequences on the whole ecosystem (Nixon 1995, 2009; Conley et al. 2009).

Eutrophication may result from a variety of anthropogenic practices, including agricultural activities, waste waters and aquacultures. Due to the dramatic decline of natural fish stocks, aquacultures are becoming increasingly important. Ecological threats, such as eutrophication and biodiversity loss, lead to current concerns about coastal aquaculture practices (Naylor et al. 2000). Especially the accumulation of organic waste products is of great concern (e.g. Cancemi et al., 2003).

Increased nutrient loading and accumulation of aquaculture derived waste products may not only stimulate (toxic) algal blooms but also result in high POM loading in coastal waters. If the sinking velocities of particles (of both, biological or anthropogenic origin) are high and their dispersal due to currents is low, the organic matter will accumulate locally on the shallow sea floors (Cromey and Black 2005). At the sea floor OM serves as electron acceptor for heterotrophic sediment bacteria and its excess can lead to hypoxic conditions, affecting benthic biodiversity (Holmer et al. 2002; Nacorda et al. 2012).

If the aquaculture derived POM is transported to adjacent ecosystems, such as seagrass beds, and coral reefs, the increased nutrient and organic matter load can negatively impact their ecosystem function (Orth et al. 2006; Hedberg et al. 2017). Since aquacultures are expected to expand, one of the major challenges is to render them more sustainable (Ahmed et al. 2017)

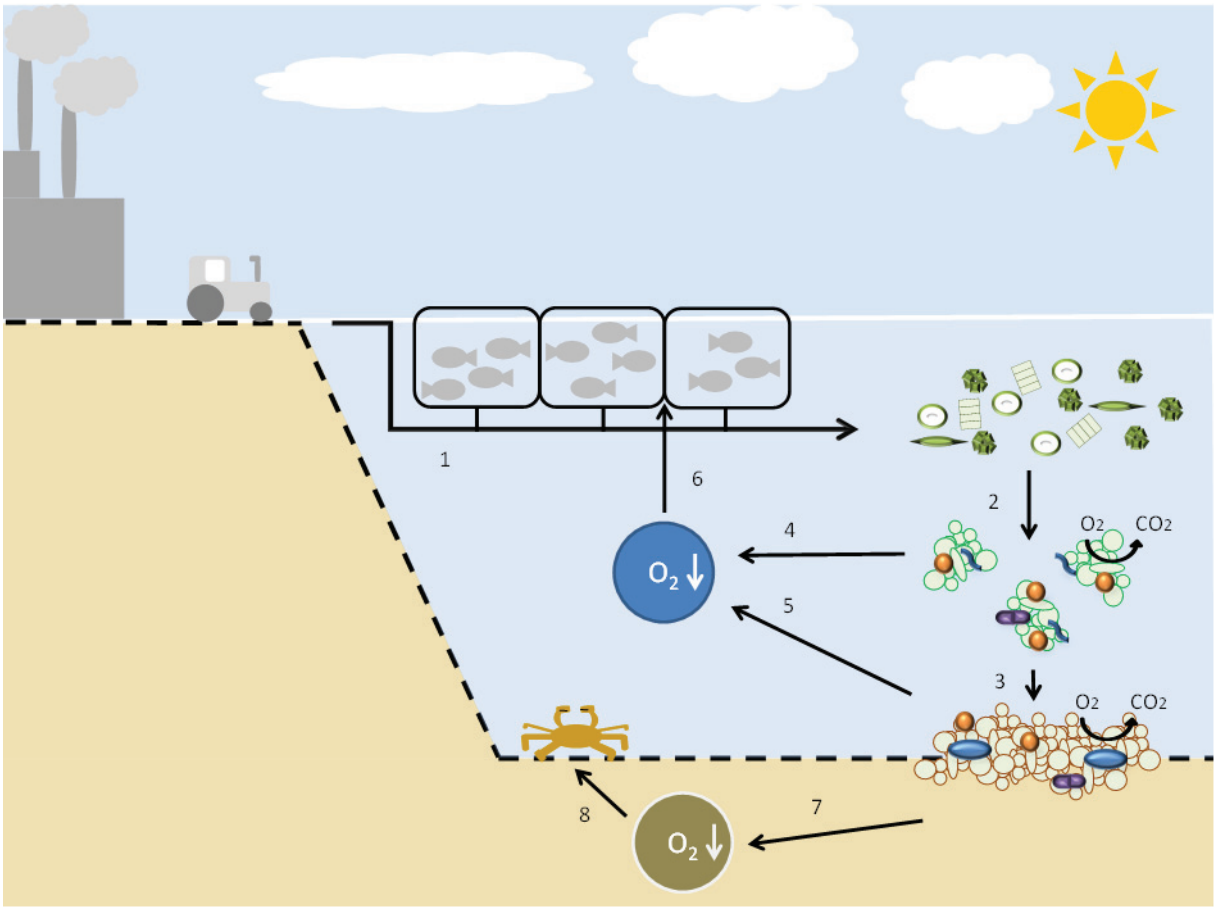

Figure 5. The process of eutrophication: Anthropogenic activities, including fish farming, leads to increased inorganic nutrient and organic matter concentrations. These nutrients stimulate the growth of phytoplankton (1). Dying phytoplankton cells aggregate and attached heterotrophic bacteria degrade the organic material, thereby consuming oxygen (2). The particulate organic matter sinks to the sea floor, where it accumulates and microbes further decompose the substrates (3). The microbial activity can result in a large drawdown of oxygen, in turn affecting reared fish (4, 5, 6). Benthic oxygen depletion leads to the depletion of macro-fauna, reducing the biodiversity and bioturbation of this habitat.

Heterotrophic bacteria in eutrophic systems

In eutrophic environments, where organic matter concentrations are generally very high, copiotrophic bacteria thrive well. In tropical aquaculture influenced eutrophic coastal ecosystems, warm water temperature can increase the organic matter turnover by heterotrophic bacteria (Iversen and Ploug 2013). Resulting high rates of organic matter remineralization by heterotrophic bacteria may contribute to low oxygen concentrations in the water column.

Furthermore, the eutrophic conditions can be mirrored in bacterial abundances, alpha diversities and communities (Yang and Kwon 2017). Hence, PA BCC may serve as indicators for aquaculture impact.

Study site II: The aquaculture influenced coastal region off Bolinao, Philippines

Asia, which produced around 90% of the global aquaculture output in 2013, is the most important continent for aquaculture productivity (Ottinger et al. 2016). In Bolinao, Philippines, intensive milkfish (*Chanos chanos*) mariculture has flourished since the 1990s but has expanded well beyond the carrying capacity of the area (Santander-de Leon et al. 2015). As a result the costal environment has become eutrophic and deteriorated (Reichardt et al. 2013). In 2005, a *Vibrio cholera* epidemic broke out in the region and fish kills occur repeatedly, coinciding with dying algal blooms and low oxygen concentrations (Azanza et al. 2005; San Diego-McGlone et al. 2008; Reichardt et al. 2013).

In order to investigate the aquaculture practices in Bolinao from a multidisciplinary perspective, the project ACUTE (AquaCUlture practice in Tropical coastal Ecosystems) has been launched. Over the past three years, a group of four scientists has worked on the following work packages (WPs): Biogeochemistry (WP1), microbiology (WP2), fish physiology (WP3) and socio-economics (WP4). This thesis contains the results of WP1, which will be put into perspective of the results of the other WPs in the general discussion.

Aims of this thesis

In **Chapter II** the BCC and the dissimilarity/ similarity between FL and PA bacteria will be investigated off the coasts of Mauritania and Senegal.

Although the upwelling region off Mauritania and Senegal plays an important role in fisheries productivity and carbon sequestration, the base of the food web, the prokayotic micorbial communities, have received little attention, yet. Studies on benthic N_2 fixation (Gier et al. 2017), microbial methanol uptake (Dixon et al. 2013) and a CARD-FISH based investigation of the BCC further offshore (Thiele et al. 2015) have been carried out. However, there is no systematic and detailed molecular characterization of the FL and PA microbial communities off Mauritania and Senegal.

Furthermore, there has been a continuing debate in the literature about whether the FL and PA bacterial fractions are generally similar (Hollibaugh et al. 2000; Ortega-Retuerta et al. 2013) or dissimilar (Acinas et al., 1999; DeLong et al., 1993; Rieck et al., 2015; Zhang et al., 2016a). Although environmental factors can explain around 25% of bulk microbial community variability, the effect of environmental parameters, such as temperature, salinity and substrate variability, on the similarity/ dissimilarity of the FL and PA fraction has not been investigated yet. Similarities between FL and PA might reflect exchange of PA bacteria between different substrates. Thus the investigation about similarities/ dissimilarities of FL and PA bacteria in water masses with different substrate availabilities could help elucidate microbial particle dynamics further.

Along the coastlines of Mauritania and Senegal, there are horizontal and vertical gradients in environmental variables and substrate availability. This makes the NW African coast an ideal model system for studying the effects of environmental variables, such as temperature, salinity and substrate availability, on the similarity/ dissimilarity of FL and PA bacterial fractions.

Therefore, the first aim of this study will be to determine the FL and PA BCC off NW Africa. Secondly, we will identify environmental drivers that affect the similarity/ dissimilarity between PA and FL bacteria.

In **Chapter III** the microbial key players in the BCP off NW Africa will be examined more closely. Along the coast of Mauritania and Senegal, seasonal upwelling and Saharan dust input lead to the natural fertilization of this system (Fischer et al. 2009). Ballasting with

Saharan dust of sinking particles originating from this productive area contributes to an efficient BCP (van der Jagt et al. 2018). Despite the importance of the Canary Current system for global carbon sequestration, little is known about the microbial key players in the BCP off NW Africa. Especially potential changes of micro- and macro PA bacterial communities as they sink through different water masses has not been studied, yet.

Therefore, in this study, we will investigate microbial BCC attached to particles in the BCP off Mauritania and Senegal more closely. Furthermore, we will examine the variability of micro- and macro-PA BCC, when these particles are subjected to different water masses.

In **Chapter IV**, the focus was on organic particles/ aggregates and their relation to coastal biogeochemistry.

In Bolinao, Philippines, aquaculture activities have caused environmental deterioration. High sedimentation rates of organic particles originating from the aquaculture effluents have led to anoxic sediments (Holmer et al. 2002, 2003). Nevertheless, the impact of mariculture on particle dynamics has received little attention. Especially microbial particle colonization and associated high respiration rates, which may contribute to recurring oxygen depletion and subsequent fish kills, have not been studied, yet. In this chapter we will investigate if organic particles may serve as indicators or aquaculture impact on the costal environment.

The first aim of Chapter IV of this thesis will be the characterization of aquaculture derived organic particles and their relation to microbial activities and coastal biogeochemistry. Secondly we will explore if particle characteristics can be used as an indicator for the quantification of aquaculture effects on the coastal environment.

Publication overview

Chapter II has already been published and Chapters III and IV are intended for publication in international peer-reviewed journals.

Chapter II: *Environmental drivers of free-living vs. particle-attached bacterial community composition in the Mauritania upwelling system*

Jennifer Bachmann, Tabea Heimbach, Christiane Hassenrück, Germán A. Kopprio, Morten Hvitfeldt Iversen, Hans-Peter Grossart and Astrid Gärdes

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Author contributions: The study was conceived and designed by JB (70%), HPG, MI and AG. The field and lab work was performed by JB (50%), TH and GC. Data analysis and interpretation was performed by JB (75%) and all other contributing authors. The manuscript was written by JB. HPG, CH, MI, GC and AG helped to improve the final manuscript.

Chapter III: *The shelf biological carbon pump off North-West Africa*

Jennifer Bachmann, Tabea Heimbach, Christiane Hassenrück, Morten Hvitfeldt Iversen, Hans-Peter Grossart and Astrid Gärdes

Manuscript in preparation

Author contributions: The study was conceived and designed by JB (70%), HPG, MI and AG. The field and lab work was performed by JB (80%) and TH. Data analysis and interpretation were performed by JB (80%), MI, HPG, AG and CH. The manuscript was written by JB. HPG, CH, MI and AG helped to improve the final manuscript.

Chapter IV: *Particles in the spotlight: a measure of aquaculture-induced impacts on aquatic ecosystems*

Jennifer Bachmann, Christiane Hassenrück, Yustian Rovi Alfiansah, Morten Hvitfeldt Iversen, Hans-Peter Grossart and Astrid Gärdes

Manuscript in preparation

Author contributions: The study was conceived and designed by JB (85%), HPG, MI, CH and AG. The field and lab work was performed by CH, YA and JB (20%). Data analysis and

interpretation were performed by JB (80%), MI, HPG, AG and CH. The manuscript was written by JB with improvements from HP, CH, AG and MI.

Contributed work:

Appendix: *Evaluating impacts of intensive milkfish aquaculture on water quality, organic matter loading, and bacterial communities in Bolinao, Philippines*

Christiane Hassenrück, Jennifer Bachmann, Inken Hanke, Chyrene Moncada, Cecilia Conaco, Morten Hvitfeldt Iversen, Hans-Peter Grossart, Astrid Gärdes

Author contributions: The study was conceived and designed by CH, HPG, MI, AG and JB (15%). The field and lab work was performed by CH, IH, CM and JB (5%). Data analysis and interpretation were performed by CH, MI, HPG, AG and JB (5%). The manuscript will be written by CH.

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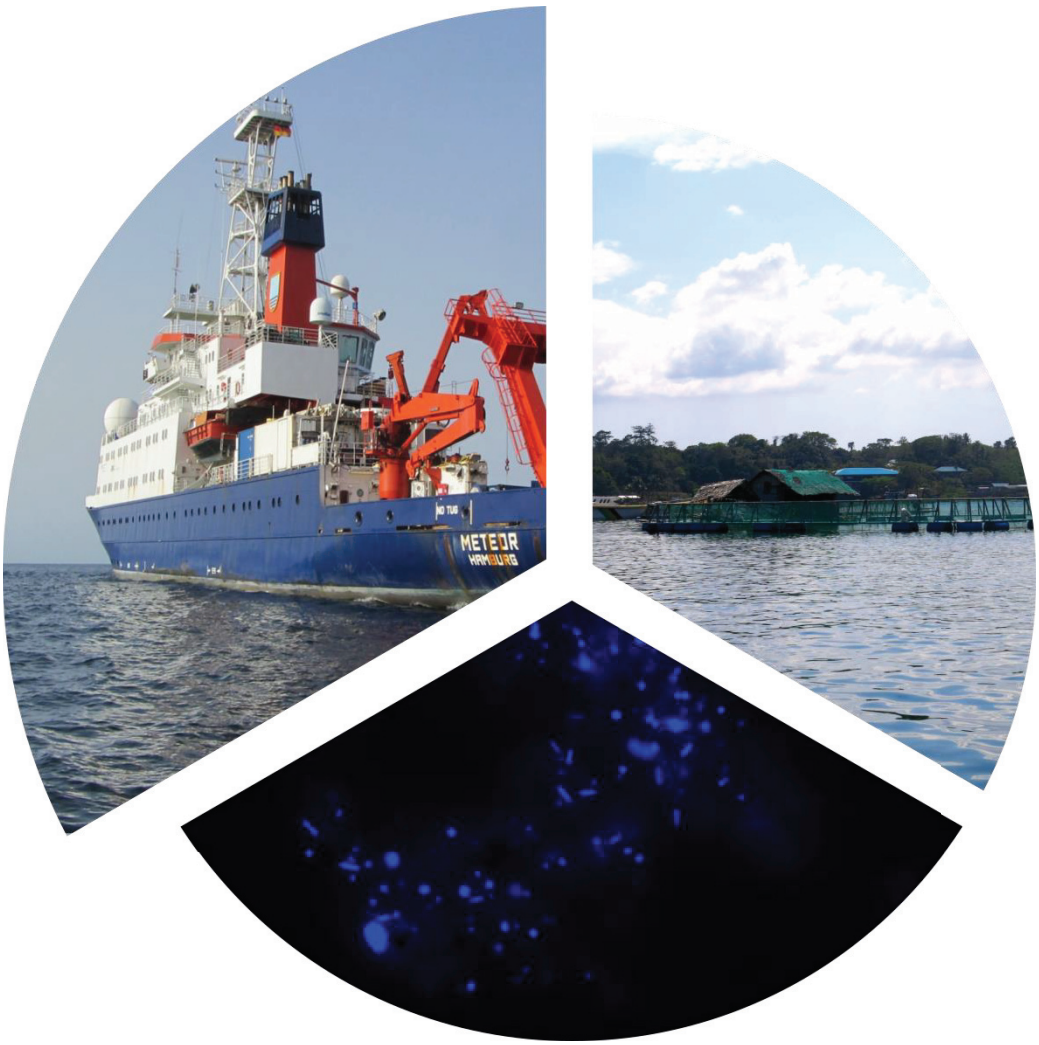
CHAPTER II



frontiers in Microbiology

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Environmental Drivers of Free-Living vs. Particle-Attached Bacterial Community Composition in the Mauritania Upwelling System

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Saharan dust input and seasonal upwelling along North–West Africa provide a model system for studying microbial processes related to the export and recycling of nutrients. This study offers the first molecular characterization of prokaryotic particle-attached (PA; >3.0 μm) and free-living (FL; 0.2–3.0 μm) players in this important ecosystem during August 2016. Environmental drivers for alpha-diversity, bacterial community composition, and differences between FL and PA fractions were identified. The ultra-oligotrophic waters off Senegal were dominated by Cyanobacteria while higher relative abundances of Alphaproteobacteria, Bacteroidetes, Verrucomicrobia, and Planctomycetes (known particle-degraders) occurred in the upwelling area. Temperature, proxy for different water masses, was the best predictor for changes in FL communities. PA community variation was best explained by temperature and ammonium. Bray Curtis dissimilarities between FL and PA were generally very high and correlated with temperature and salinity in surface waters. Greatest similarities between FL and PA occurred at the deep chlorophyll maximum, where bacterial substrate availability was likely highest. This indicates that environmental drivers do not only influence changes among FL and PA communities but also differences between them. This could provide an explanation for contradicting results obtained by different studies regarding the dissimilarity/similarity between FL and PA communities and their biogeochemical functions.

Keywords: prokaryotes, biodiversity, microbial ecology, alpha diversity, Bray Curtis dissimilarity, temperature, salinity, 16S rRNA Illumina amplicon sequencing

INTRODUCTION

Bacteria and archaea, the unseen majority (Whitman et al., 1998), carry out important steps in the biogeochemical cycling of carbon and nutrients, and thus are pivotal for the functioning of marine ecosystems. Many heterotrophic bacteria hydrolyze particulate into dissolved organic matter (OM) (Azam and Malfatti, 2007), which is further hydrolyzed into small molecules for direct uptake by

heterotrophic microorganisms. Furthermore, the microbial loop (Azam et al., 1983) converts waste products and other OM into bioavailable organic and inorganic nutrients and subsequently releases them into the surrounding water supporting new biomass production. Thereby, heterotrophic bacteria provide essential nutrients for phytoplankton primary production, which forms the base of any aquatic food web.

Generally, pelagic bacteria can be categorized as truly free-living (FL), truly particle-attached (PA), and bacteria alternating between the two lifestyles (Grossart, 2010). The truly FL bacteria spend their whole life-cycle suspended in the water column, whereas truly PA bacteria remain predominantly associated with various sorts of particles. In contrast, alternating bacteria can constantly attach and detach from particles and hence move between both life-styles. These differences in life-styles are linked to metabolic differences, e.g., PA bacteria generally have a higher capacity to degrade polymeric OM than FL bacteria (Lyons and Dobbs, 2012). Therefore, it is important to distinguish between bacterial life-styles for a better understanding of bacterial community composition (BCC), dynamics, and functions in the ocean.

Particle-attached bacteria are involved in the degradation of sinking particulate OM and thereby affect the efficiency of the biological carbon pump: Previous studies have shown that microbial communities attached to sinking particles typically possess carbon-specific respiration rates of around 0.1–0.2 d^{-1} (Ploug et al., 1999). Hence, their respiration and activity can importantly reduce the amount of organic carbon that is exported to below the thermocline. In this context, it has since been recognized that the capacity for particulate OM degradation depends on the BCC (Enke et al., 2018). Additionally, it has been shown that sinking particles and their attached microbial communities contribute to the vertical connectivity of the BCC in the ocean (Mestre et al., 2018). Thus to understand the functional role of bacteria, knowledge on PA community composition is of profound importance in marine regions characterized by a high carbon export.

Eastern boundary currents, such as the Canary Current, are among the most productive marine regions of the world (Carr, 2002). By taking up large amounts of carbon, the Canary Current system plays an important role in the global export of organic carbon (Arístegui et al., 2009; Fischer et al., 2016). In the southern part of the Canary Current ecosystem, upwelling of nutrients along the coasts of Mauritania and Senegal supports global fisheries by providing suitable spawning grounds for many economically important fish species (Arístegui et al., 2009). Upwelling and surface currents in this region are influenced by changes in trade winds and the migration of the intertropical convergence zone toward the relatively warmer hemisphere between 2°N during boreal winter and 9°N during summer (Schneider et al., 2014). Additionally, Saharan dust input leads to the natural fertilization of this system (Fischer et al., 2009).

Despite the region's importance for fisheries and the global carbon cycle, the prokaryotic microbial community off the coast of Mauritania and Senegal has received little attention. Dinitrogen fixation by benthic cyanobacteria (Gier et al., 2017) and microbial methanol uptake (Dixon et al., 2013) have been assessed off Mauritania but only one CARD-FISH-based investigation of the whole BCC has been carried out further offshore (Thiele et al., 2015). They observed highest similarities between PA bacteria at depth and FL bacteria from the deep chlorophyll maximum (DCM). High particle export, frequent changes in the intensity of the year-round upwelling and the natural fertilization with Saharan dust (Fischer et al., 2016), render this region a model system for studying microbial processes related to the export and recycling of inorganic nutrients and carbon in the ocean.

Since the micro-scale heterogeneity varies among aquatic habitats, the distribution of specific bacterial species in the ocean is not uniform (Stocker, 2012), whereby the BCC depends on both abiotic (e.g., temperature, pH, etc.) and biotic factors (e.g., algal blooms, zooplankton feces, etc.). It has been demonstrated that in general about 25% of the variation microbial community composition can be explained by environmental parameters (Hanson et al., 2012). Previous studies have correlated bacterial community structures with environmental parameters, such as salinity (Rieck et al., 2015), quantity and quality of inorganic nutrients and OM (Ortega-Retuerta et al., 2013), particle composition (although unsuccessfully; Zhang et al., 2016) as well as chlorophyll *a* (chl*a*) and temperature (Kan et al., 2006). FL and PA bacteria, however, seem to be differentially affected by bulk water parameters, e.g., the larger the particle, the more insensitive the attached community is to changes in abiotic water parameters (Yung et al., 2016). Thereby, the PA community was mainly influenced by substrate availability and particle quality (Yung et al., 2016). Very few studies have assessed the effects of the latter on the similarity/dissimilarity of FL and PA communities, and there has been some debate in the literature about whether FL and PA bacteria are generally similar (Hollibaugh et al., 2000; Ortega-Retuerta et al., 2013) or dissimilar (DeLong et al., 1993; Acinas et al., 1999; Rieck et al., 2015; Zhang et al., 2016).

Along the coastlines of Mauritania and Senegal natural horizontal and vertical gradients in environmental variables and substrate availability exist, rendering the North–West African coast an ideal models system for those studies. In the surface waters from Mauritania to Senegal, horizontal gradients of decreasing salinity and inorganic nutrients and increasing temperature result from riverine water discharge and precipitation. Vertically, substrate availability changes from the oligotrophic surface waters off Senegal, over the more productive DCM to 200 m depth, where life almost exclusively relies on sinking OM from the sunlit ocean. The DCM is a prevalent structure of tropical marine environments, occurs at around 20–100 m depth and is characterized by increased concentrations of chl*a*. The DCM often coincides with high primary productivity as environmental conditions are ideal for phytoplankton growth (Eppley et al., 1988; Raimbault et al., 1993; Cullen, 2015). Thus, substrate availability for bacteria may be high at the DCM. However, so far the effect of substrate availability on the BCC in natural environments has received little attention. During the Meteor M129 cruise to the Golf d'Arguin (in Northern Mauritania) and Sine Saloum (Senegal, **Figure 1**) in July to August 2016, we investigated the BCC off Mauritania and Senegal and the effect of substrate availability on the similarity

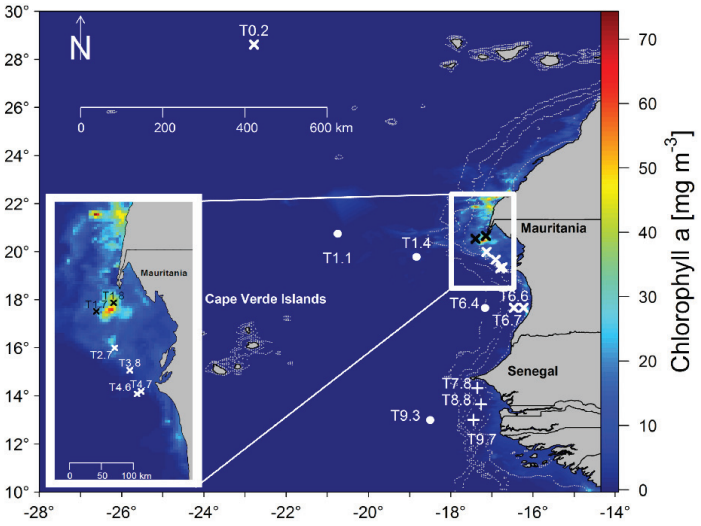

FIGURE 1 | Sampling stations for this project during M129. Sampling was carried out at 16 stations at the surface only (x) surface and DCM (+) and from surface, over DCM to 200 m depth (•). The figure in the lower left corner is an enlargement of the sampling region off Northern Mauritania. Stations T1.7 and T1.8 (black) were situated in the core of the upwelling area, while station T1.4 was within in an anticyclonic eddy. The map was produced from Level 3 Modis Aqua satellite imagery data on monthly chlorophyll a concentrations obtained from https://oceandata.sci.gsfc.nasa.gov/MODIS-Aqua/.

between FL and PA bacteria by addressing the following research questions:

1. What is the FL and PA microbial community composition off NW Africa, and which conclusions can we draw with regard to the role of PA bacteria in the degradation of organic particles?
2. What are the effects of environmental parameters and substrate availability on the similarity/dissimilarity of FL and PA bacterial communities in this region?

We hypothesize that bacteria, which are adapted to (ultra-)oligotrophic conditions, predominate off Senegal, while the upwelling and potential formation of phytoplankton blooms may stimulate taxa adapted to more mesotrophic conditions off the North–West African coast. Temperature is assumed to be the main environmental driver for changes in overall BCC, and we hypothesize that environmental factors, such as temperature, salinity, and substrate availability, also affect the differences between FL and PA communities, but to a different extent. As we expect the highest substrate availabilities at the depths of the DCM, we hypothesize that FL and PA communities will be most similar there.

MATERIALS AND METHODS

The M129 cruise started from the Azores, on July 30 and finished on the Cape Verdes on August 25, 2016. Sampling was carried out along several transects off the coast of Mauritania and Senegal (**Figure 1**). Sampling stations were distributed over different oceanic regions, including the shelf break and shallow (<50 m) waters closer to the coast. More information about the respective M129 cruise stations (see **Supplementary Table 1**) is available under https://www.pangaea.de/ddi?retr=events/Meteor_1986/M129.retr&conf=events/CruiseReportHTML.conf&title=Station+list+of+cruise+M129&format=html. Water samples were obtained using a SBE32 rosette water sampler with 24 × 10L Niskin bottles (s/n 0342) from 16 stations over 3 depths: 16 surface samples (ca. 5 m), 7 DCM samples, and 4 samples from 200 m depth (below the thermocline). Water samples were immediately filtered through a 125-μm mesh in order to exclude larger organisms, such as zooplankton. The filtrate was collected in polyethylene canisters and processed under laboratory conditions within 2–3 h.

Physicochemical parameters (temperature, salinity, chl*a*, and turbidity) were measured *in situ* using the Rosette water sampler, which was equipped with a Seabird_electronics SBE9 CTD (s/n 0572) and a digiquartz pressure sensor (s/n 75760) using a double sensor setup.

Chemical Parameters

Particulate organic carbon (POC) samples were taken at all surface stations. Between 1000 and 5000 mL of marine water were filtered through pre-combusted (450°C for 4 h) and pre-weighted 47 mm GF/F filters (Whatman, Dassel, Germany). Filters were dried at 40°C for at least 24 h and total carbon (TC) and total nitrogen (TN) concentrations were measured on an Elemental Analyzer (EA-3000, EuroVector, Italy). The organic carbon fraction (C_{-org} or POC) was measured after acidification

of the filter with 1 N HCl to remove the inorganic carbon. CN ratios were calculated from C_{-org} and TN.

Dissolved inorganic nutrient (NUT) samples were taken in technical triplicates from the POC filtrate and frozen at −20°C until analysis on board. Using a continuous segmented flow autoanalyzer (San System, Skalar, Netherlands) the concentrations of five NUT, i.e., nitrate, nitrite, ammonium, phosphate, and silicate, were determined after Grasshoff et al. (1999).

Technical triplicates of dissolved organic carbon (DOC) concentrations were also obtained from the POC filtrate and preserved frozen at −20°C. Acidified DOC samples were analyzed using the high-temperature combustion method (Dafner and Wangersky, 2002) with a TOC-VCPH autoanalyzer (Shimadzu, Mandel, Canada).

Plankton Counts

Plankton samples for quantitative analysis were taken at selected surface (T1.1, T0.2, T3.8, T1.4, T4.6, T8.8), DCM (T1.1, T1.4, T7.8, T9.3), and 200 m stations (T1.1, T9.3). Samples were filtered through a 125-μm mesh in order to remove larger organisms and fixed with formalin (2% v/v final concentration). For analysis, 100 mL of the fixed sample were filled into an Utermöhl chamber. Cells were counted after overnight sedimentation at 400× magnification and across 50 random fields. The identified cells were classified into the following plankton groups: diatoms, haptophytes, dinoflagellates, non-flagellated chlorophytes, and other flagellates.

Bacterial Enumeration

Water samples (50–150 mL) were fixed with formaldehyde (2% v/v) and stored at 4°C for 48 h. PA and FL bacteria were separated via sequential filtration through 3.0 and 0.2 μm (Kegler et al., 2017) Nuclepore TrackEtch polycarbonate membranes (Whatman, Dassel, Germany), respectively. Filters were air-dried and frozen until microscopic analysis (Rieck et al., 2015). 4′,6-Diamidino-2-phenylindole (DAPI; Thermofisher Scientific Inc., Waltham, MA, United States) was diluted in a 3:1 mounting solution (made of Citifluor AF mounting medium; Citifluor Ltd., London, United Kingdom) and Vecta shield (Vector Laboratories Inc., Burlingame, CA, United States) to a concentration of 1 μg mL^{-1}. The DAPI/mounting medium solution was directly added onto the filter. FL bacteria were enumerated by using an automatic microbial cell enumeration system. A multipurpose fully automated microscope imaging system (MPISYS) was used for the refined image acquisition. Image selection, cell determination, and enumeration were carried out using the ACMEtool2.0 (Bennke et al., 2016). PA bacteria were manually enumerated with an epifluorescence microscope "Axioskop 40" (Zeiss, Jena, Germany) at 1000× magnifications (Grossart et al., 2005). A minimum of 40 grids (grid size: 15,625 μm^2) was counted.

Molecular Analysis of the Microbial Community

FL and PA bacteria were separated as described under "*Bacterial enumeration*" (for filtered volumes see **Supplementary Table 2**). Filters were frozen on board and transported at −80°C. Storage at the ZMT until further processing was at −20°C. DNA extraction was carried out after Nercessian et al. (2005). Briefly, the phenol–chloroform–isoamylalcohol extraction protocol was adapted for planktonic bacteria as previously proposed by Rieck et al. (2015). Cetyltrimethyl ammonium bromide (CTAB) was used as a complexing agent for polymeric substances. For all samples, the second chloroform–isoamylalcohol washing treatment was skipped. DNA extracts were sent to LGC genomics (Berlin, Germany) for amplicon sequencing of the microbial community. The primer pair Bakt_341F (5′-TCCTACGGGGGCWGCAG-3′) and Bakt_805R (5′-TGACTACHVGGGTATCTAAKCC-3′) was used to target the hypervariable regions V3–V4 of the bacterial 16S rRNA gene (Klindworth et al., 2013). Sequencing was performed on an Illumina MiSeq using V3 Chemistry (Illumina) in a 2× 300 base pair paired-end run.

Bioinformatic Analyses

Demultiplexing and the removal of primer sequences from the raw paired-end reads with *cutadapt* (Martin, 2011) were performed by LGC genomics. Sequences were further analyzed according to Hassenrück et al. (2016): Sequences were quality trimmed with a sliding widow of four bases and an average quality of 15 using *trimmomatic* v.033 (Bolger et al., 2014). Using *PEAR* v0.9.6 (Zhang et al., 2014), sequences were merged, and *swarm* v2.1.1 was applied to cluster operational taxonomic units (OTUs) (Mahé et al., 2014). Taxonomic classification with SILVA 128 was carried out using the SILVA-ngs pipeline (Quast et al., 2013). Singletons, doubletons, chloroplasts, mitochondria, and OTUs unclassified on phylum level were excluded.

Based on the 16S gene of the chloroplast the presence/absence of eukaryotic phytoplankton genera was inferred (Schmidt et al., 1991; Needham et al., 2017). Chloroplast 16S sequences were aligned against a customized reference database only containing chloroplast sequences from cultivated organisms obtained from the NCBI refseq database (date accessed: June 27, 2017). Sequences with >93% similarity were filtered out for further analysis and the taxonomic path for the best hit was extracted. A detection threshold of at least 10 sequences was chosen to ensure that the sequenced chloroplasts were really present. Omission of non-phytoplankton lineages and manual curation of the taxonomic path ensured optimal phytoplankton characterization based on the 16S region of chloroplasts.

Data Archiving

In compliance with the Minimal Information about any (X) Sequence (MIxS) standard (Yilmaz et al., 2011), the raw data of demultiplexed and primer-clipped sequences were deposited at the European Nucleotide Archive (ENA; Toribio et al., 2017) using the data brokerage service of the German Federation for Biological Data (GFBio; Diepenbroek et al., 2014). They are accessible under PRJEB26997.

Statistical Analyses

All statistical analyses were run in R studio using the core distribution (R-Core-Team, 2015) and additional packages, such as *vegan* (Oksanen et al., 2015) and *gplots* (Warnes et al., 2016).

Environmental parameters were displayed in a principal component analysis (PCA). The three missing values of turbidity, fluorescence, and O_2 were substituted by the means of all other samples of the respective parameter. Based on the PCA, the samples were classified into one of the following categories: surface upwelling (Sur-UW), surface oligotrophic (Sur-oligo), DCM, 200 m, as well as eddy surface (Eddy-sur), eddy DCM (Eddy-DCM), and Eddy-200 m samples. Eddy samples were too different from the others in the respective category but since only one eddy was sampled the Eddy categories had to be excluded from any statistical analyses.

PERmutational Multivariate Analysis Of Variance (PERMANOVA) and Analysis Of SIMilarity (ANOSIM) based on Jaccard dissimilarity were used to test for differences in phytoplankton presence/absence between the categories.

Differences between FL and PA bacterial cell numbers and alpha diversity were assessed with paired *t*-tests. Alpha diversity was assessed using the Inverse Simpson Index calculated without prior rarefying to equal library sizes as rarefaction curves based on this index were saturated at the obtained sequencing depths (Chao et al., 2014). To test for significant differences in alpha diversity among FL and PA, two alternatives were tested: (i) differences occurring between categories of samples identified in the PCA using ANOVA and if applicable Tukey's test, and (ii) correlation with environmental parameters using a linear model. For all regression-based analyses, collinearity between predictor variables was checked and avoided so that only temperature, salinity, fluorescence, ammonium, and DOC remained.

Pairwise BC dissimilarities were used for cluster analysis (unweighted pair-group method using arithmetic average, UPGMA). Ordination of similarities within each separate fraction was carried out using non-metric multidimensional scaling (NMDS) on relative abundances of FL and PA OTUs. In order to test differences in beta diversity trends between FL and PA, a Procrustes analysis was run on these NMDS ordinations.

Redundancy analysis (RDA) was performed with centered log-ratio-transformed sequence counts of both FL and PA fractions, separately. Variation partitioning was used to assess the contribution of each of the environmental parameters in the RDA models, while accounting for the variability explained by the others (pure effects). Forward model selection was conducted to identify the best-fitting RDA models based on the minimum Akaike Information Criterion (AIC).

RESULTS

Physical and Chemical Environmental Parameters

Physicochemical environmental parameters were ordinated in a PCA (**Figure 2**) and all raw values are archived at PANGAEA[1] and **Supplementary Table 1**. In the PCA, the first two principal components explained 62% of the variability in the data set. PC1 was mainly influenced by nutrients (except for ammonium), which were positively correlated with PC1, and physico-chemical parameters related to different water masses, which showed a negative correlation with this PC. PC1 was an indicator for water depth, generally ordinating the deeper stations, which were colder and richer in nutrients, in the positive range. Apart from three upwelling stations (T1.8, T2.7, and T3.8) all surface stations, characterized by higher temperatures and lower nutrients, clustered in the negative range of PC1. PC2 was an indicator for upwelling and was positively correlated with fluorescence and turbidity, as well as DOC and ammonium, i.e., variables indicating productivity.

Three of the four 200 m samples clustered closely together. T1.1 was not grouped closely with the other 200 m samples, indicating its origin from a different water mass. An oxygen minimum with 7 μmol L^{-1} at 30 m depth, the direction of rotation and density structure (obtained from CTD profiles and acoustic doppler current profiler; data unpublished) suggested that station T1.4 was located within an anticyclonic mode water eddy (ACME). The Eddy-DCM station was very different from the remaining loosely clustering DCM stations. At station T1.4, turbidity and fluorescence were highest (0.2 and 6.1 mg m^{-3}, respectively) among all DCM samples and were within the range of the Sur-UW samples. Station T1.4 also had the highest N:P ratio (14.5) while all other DCM stations showed ratios between 2 and 12.7.

Six surface stations were characterized by elevated chl*a* concentrations (**Figure 1**) together with nitrate concentrations of >2 μM and were thus categorized as upwelling samples. Station T2.7 was found on the projectory line of the ammonium vector because it had the highest ammonium concentration of all stations (**Figure 2**). Temperatures of the Sur-oligo stations were warmer and increased from Mauritania (approximately 23°C) toward Senegal (approximately 29°C). Salinities of Sur-oligo stations ranged from 35 to 37.1 with the lowest and highest values off Senegal (T3.9) and offshore off Mauritania (T0.2), respectively.

Neither of the Sur-UW nor the Sur-oligo surface samples had N:P ratios (all <11) close to Redfield ratio. Nutrient concentrations indicated that the upwelling stations were at most mesotrophic and Sur-oligo stations were oligo- to ultraoligotrophic.

Phytoplankton Composition

Microscopic analysis (**Supplementary Figure 1**) of phytoplankton communities at 14 out of 27 samples revealed concentrations between 10,400 cells L^{-1} (at T1.1 200 m) and more than 10^6 cells L^{-1} (at the Eddy surface station T1.4). The lowest surface numbers were observed at T1.1 with approximately 20,000 cells L^{-1}. In general, cyanobacteria and haptophytes were the dominant phytoplankton groups in this area. Diatom cell numbers did not exceed 45,000 cells L^{-1} but were the most diverse phylum (with 20 genera). They included, e.g., *Thalassiosira* and *Chaetoceros*. *Emiliania* and *Phaeocystis* were the only two detected genera of haptophytes.

[1] https://doi.pangaea.de/10.1594/PANGAEA.889977

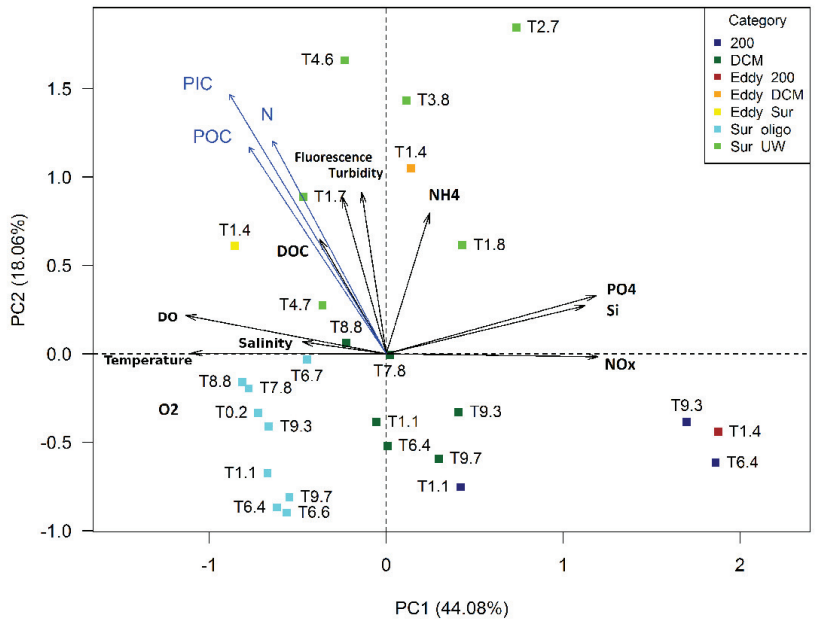

FIGURE 2 | *Principal component analysis (PCA)* of environmental variables measured at the sampling stations (type II scaling). Particulate organic/inorganic carbon (POC/PIC) and particulate nitrogen (N) concentrations were only available from surface waters. They were added to the PCA using *envfit*. DO, dissolved oxygen.

The presence/absence of phytoplankton genera based on chloroplast sequences (**Supplementary Figure 2**) indicated spatial trends over the sampling area: Few to no genera were found in the 200 m samples. Upwelling samples clustered together in the heat map, indicating similar phytoplankton communities. Phytoplankton counts at the two Sur-UW stations T3.8 and T4.6 revealed that coccolithophores followed by diatoms and other flagellates were the dominant plankton groups in these samples. Although the classification of samples into the four sample categories was able to significantly explain a small proportion of phytoplankton community variation (PERMANOVA, $F_{(2,21)} = 1.84$, $R^2 = 0.15$, $p = 0.032$), this effect was not sufficient to result in well-separated communities (ANOSIM, $R = 0.05$, $p = 0.3$).

Cell Numbers of Bacterial Fractions

Abundances of FL bacteria were two to three orders of magnitude higher than those of PA (**Figure 3**). A paired *t*-test confirmed significant differences between FL and PA bacterial abundances (paired *t*-test: $t = 8.95$, $df = 26$, $p < 0.001$). Lowest cell numbers (**Figure 3**) were generally found in the 200 m samples, although the increase of cell numbers toward the surface stations was more pronounced in the FL fraction. Highest FL cell numbers ($>4 \times 10^6$ cells mL^{-1}) were found at the coastal and nutrient-rich Sur-UW station T3.8. The lowest PA numbers, with <3000 cells mL^{-1}, were measured at the deep T1.1 200 m and the saline T0.2 offshore stations, while highest PA bacterial abundances ($>9 \times 10^5$ cells mL^{-1}) were found at the more nutrient-rich and coastal surface stations T4.6 and T4.7.

Alpha Diversity of Bacterial Fractions

No significant differences in alpha diversity were found between FL and PA (paired *t*-test: $t = -1.07$, $df = 26$, $p = 0.3$). In both fractions, alpha diversity tended to increase with depth. Lowest alpha diversity was found in the Sur-oligo samples. The categories significantly explained underlying patterns in alpha diversity of both FL (ANOVA, $F_{(3,20)} = 7.79$, $p = 0.001$) and PA bacteria (ANOVA, $F_{(3,20)} = 8.35$, $p < 0.001$). Multiple pairwise comparisons indicated that the alpha diversity of FL bacteria at 200 m was significantly different from all other categories (**Supplementary Table 3**), while in the PA fraction only 200 m and surface communities differed in alpha diversity (**Supplementary Table 4**). Temperature was identified as the best predictor for explaining differences in alpha diversity in the FL (ANOVA, $F_{(1,22)} = 22.32$, $p < 0.001$) and PA fraction (ANOVA, $F_{(1,22)} = 8.58$, $p = 0.008$).

Bacterial Community Composition (BCC)

The most common bacterial phyla in this study were Proteobacteria (42%), Cyanobacteria (20%), Bacteroidetes (15%), Actinobacteria (12%), Planctomycetes (5%), Verrucomicrobia (2%), and Marinimicrobia (1.8%).

Actinobacteria, especially the OM1 clade, were typically found among the FL bacteria and shifted from the OM1 group (present in surface and DCM samples) to the family Sva0996 marine group

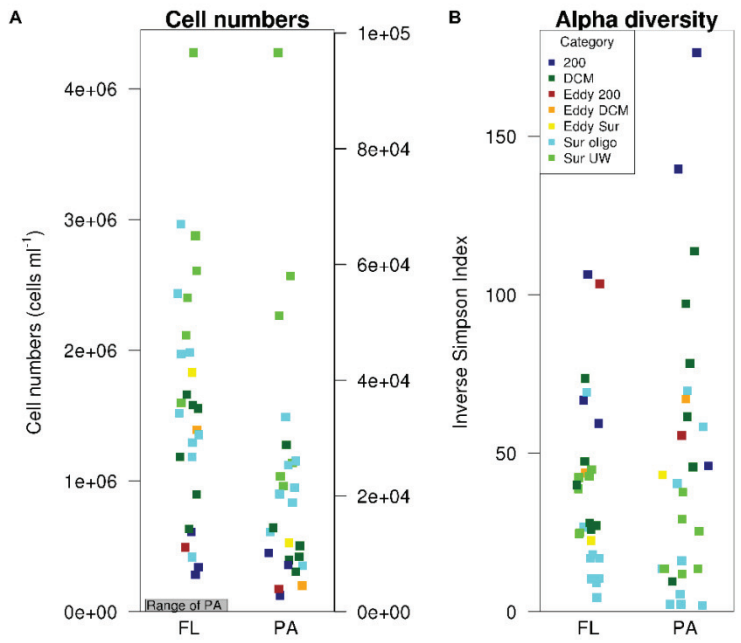

FIGURE 3 | FL and PA cell numbers and inverse Simpson index.

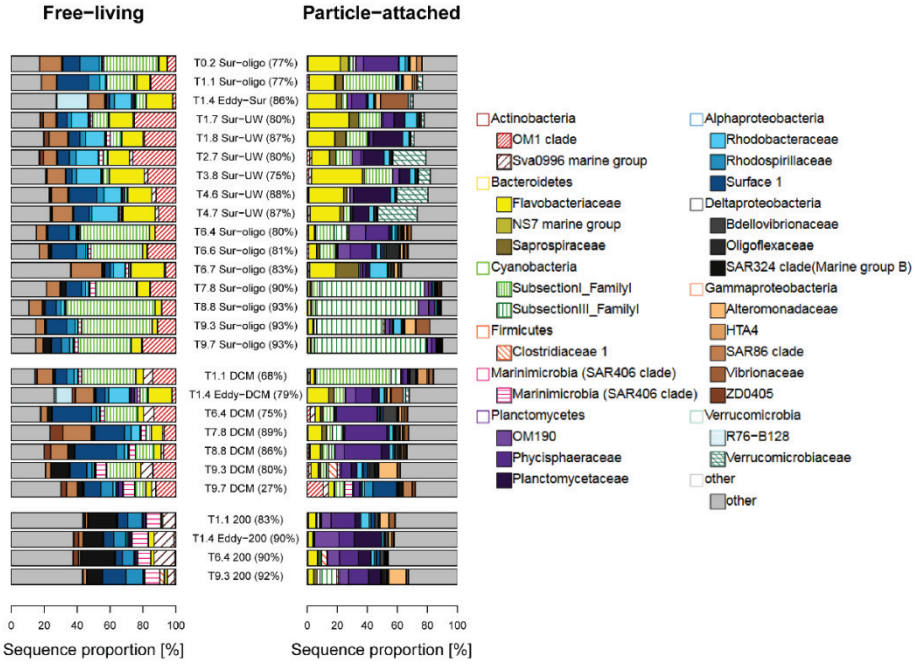

FIGURE 4 | Taxonomic compositions (family level) of FL and PA bacteria. Bray Curtis dissimilarity coefficients are given in brackets.

at 200 m (**Figure 4**). Bacteroidetes, mainly Flavobacteriaceae and Alphaproteobacteria, occurred in both FL and PA fractions. Bacteroidetes dramatically decreased in their relative abundances with depth, especially in the PA fraction off the coast of Senegal. Cyanobacteria had high relative abundances in the surface stations off Senegal. Different types of cyanobacteria dominated the FL and PA communities in the surface stations off Senegal. The FL fraction contained high relative abundances of cyanobacteria belonging to subsection I (e.g., *Synechococcus* and *Prochlorococcus*), while the PA fraction was largely dominated by cyanobacteria belonging to subsection III (mainly *Oscillatoria*). The relative abundances of Cyanobacteria decreased with increasing depth. Marinimicrobia and Planctomycetes tended to increase with depth in the FL and PA fraction, respectively. The family Planctomycetaceae was enriched in the Sur-UW samples, while other families of the same phylum prevailed in the remaining samples (**Figure 4**). Rhodobacteraceae and Flavobacteriaceae increased in relative abundances in the FL fraction of the upwelling samples. Gammaproteobacteria were represented in both FL and PA fractions, although with different families. Deltaproteobacteria had higher relative abundances at 200 m compared to the surface samples. The highest relative abundances of Verrucomicrobia in this study were found in the Sur-UW samples (from T2.8 to T4.7).

Beta Diversity and SIMPER

Unweighted pair-group method using arithmetic average (**Supplementary Figure 3**) clearly separated FL and PA communities with the exception of one PA sample (T9.7-DCM), which clustered with the FL fraction. Pairwise Bray Curtis dissimilarities between 67% and 93% confirmed that FL and PA communities were generally very different. In particular, OTUs of Actinobacteria (OM1 clade) and Cyanobacteria [*Prochlorococcus*, *Synechococcus* (both FL), and *Oscillatoria* (PA fraction)] contributed to the observed differences between FL and PA communities at the surface (**Supplementary Figure 4**). Off the coast of Senegal (T7–T9) they explained between 40% and 60% of the detected dissimilarity, while off Mauritania they explained less. Dominance of individual OTUs decreased (as alpha diversity increased) with depth. OTUs of Delta- and Gammaproteobacteria were found among the OTUs explaining the detected differences at depth.

Procrustes analysis carried out on separate NMDS ordinations of FL and PA communities (**Supplementary Figures 3B,C**) suggested that although FL and PA communities might consist of different taxa, their beta diversity patterns were congruent (correlation = 0.8; m12 squared = 0.3529; $p = 0.001$).

Environmental Drivers Shaping FL and PA Bacterial Communities

The RDA model only using temperature as predictor variable was best suited to explain differences in BCC for the FL fraction (RDA, AIC = 202.79, adjusted $R^2 = 0.24$, $F_{(1,22)} = 8.24$, $p = 0.001$). For PA bacteria, temperature [adjusted $R^2 = 0.082$, $F_{(1,21)} = 3.07$, $p = 0.001$] and ammonium [adjusted $R^2 = 0.056$, $F_{(1,21)} = 2.41$, $p = 0.001$] significantly explained the underlying patterns [RDA, adjusted $R^2 = 0.131$, $F_{(2,21)} = 2.74$, $p = 0.001$].

Pairwise Bray Curtis dissimilarity coefficients were used to assess changes in the differences between FL and PA communities among categories and environmental parameters. Although insignificant (Kruskal–Wallis, $X^2 = 3.45$, $df = 2$, p-value = 0.18), there was a trend indicating changes in Bray Curtis dissimilarities between FL and PA communities with depth (**Figure 5A**): While PA and FL communities were most similar at the DCM, Bray Curtis dissimilarities increased to around 90% at 200 m, suggesting major differences between PA and FL communities in deeper water masses. Among surface samples, differences in Bray Curtis dissimilarities existed, even though the surface categories did not explain the underlying changes. While we had insufficient statistical power to test for any significant correlations (**Figure 5D**), there was an indication that temperature and salinity correlate with surface Bray Curtis dissimilarities (**Figures 5B,C**). Especially, Bray Curtis dissimilarities of surface oligotrophic samples alone had higher correlations with both salinity ($r = -0.77$) and temperature ($r = 0.83$). Collinearity between temperature and salinity was low ($r = -0.29$) but there was a correlation between NO_x^- and temperature ($r = -0.73$) among the surface samples.

DISCUSSION

This study provides the first detailed phylogenetic characterization of the BCC off the coast of Mauritania and Senegal. Knowledge about the BCC and especially the PA fraction is the basis for further studying and understanding microbial processes related to the export and recycling of OM in this economically important upwelling system. Our results indicated that differences between FL and PA bacterial communities were simultaneously influenced by environmental parameters and substrate availability.

Environmental Variables, Phytoplankton, and Bacterial Communities Differ in the Upwelling Region

Surface upwelling and (ultra-)oligotrophic sampling stations have been differentiated using chl*a* and nitrate concentrations. During the boreal summer/autumn, upwelling off NW Africa only occurred North of 21°N as supported by satellite imagery (**Figure 1**) during our sampling campaign, and water masses most likely had a South Atlantic origin (Schütte et al., 2016). Stations T1.7 and T1.8 were situated at the southernmost tip of the upwelling region. Stations T2.8, T3.8, T4.6, and T4.7 were further south, but due to similar fluorescence values to T1.7 and T1.8, they were also characterized as upwelling samples. Dust input is also an important nutrient source, especially of phosphorous, iron, and other micro- and macronutrients, in this region (Bory and Newton, 2000; Iversen et al., 2010; Lekunberri et al., 2010; Pohl et al., 2011) and may have contributed to elevated nutrient concentrations. The remaining surface samples were characterized by lower fluorescence values. Increasing temperatures (distance to cold upwelling water and proximity to

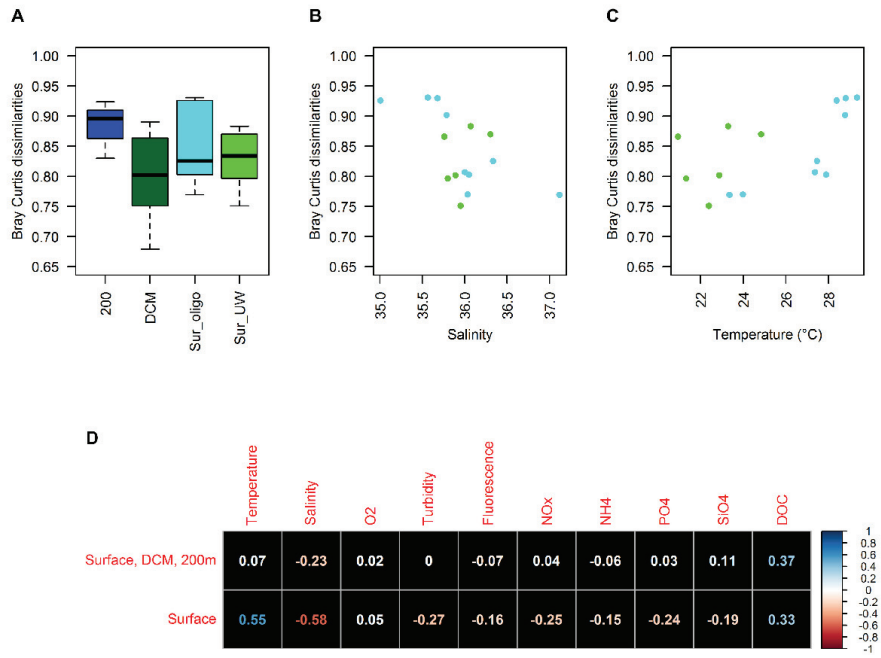

FIGURE 5 | Patterns of pairwise Bray Curtis dissimilarities between FL and PA communities: Differences between sample categories **(A)**, correlation with salinity **(B)** as well as temperature **(C)** for surface samples (especially Sur-oligo), correlation with all observed environmental parameters **(D)**.

equator) and decreasing salinity (heavy rainfall and proximity to river discharge) occurred from North to South. These Sur-oligo samples have diverse origins, from offshore waters off Mauritania to coastal waters off Senegal.

Phytoplankton communities were dominated by cyanobacteria and coccolithophores, with diatoms peaking at the upwelling stations and T0.2. The dominance of generally tiny phytoplankton cells (coccolithophores and cyanobacteria) may result from low silicate concentrations (Fischer et al., 2009) combined with the oligotrophic conditions, in which smaller cells have an advantage by being able to take up nutrients more efficiently than larger cells (Irwin et al., 2006). The bacterial communities in the ultra-oligotrophic waters off Senegal were characterized by higher relative abundances of Cyanobacteria. There, the ability to fix nitrogen may give cyanobacteria competitive advantages (Carpenter and Price, 1976).

The BCC in the upwelling area off Mauritania was dominated by Alphaproteobacteria, Bacteroidetes, Planctomycetes, and Verrucomicrobia. Planctomycetes, Verrucomicrobia, and some Bacteroidetes (e.g., *Winogradskyella*) are known to be particle-degraders and can therefore be expected to represent key players in the recycling of carbon and other nutrients. The bacterial communities in ultra-oligotrophic waters off Senegal were characterized by higher relative abundances of Cyanobacteria. Additionally, low nutrient conditions and light may have stimulated the growth of FL proteorhodopsin-containing Flavobacteria (Williams et al., 2012). Together, this may explain why Bacteroidetes are present in both fractions (although represented by different genera) and why they tend to occur at higher relative abundances only in surface waters.

FL and PA Communities Are Influenced by Environmental Parameters

Many studies have demonstrated a significant correlation between BCC and environmental parameters (Hanson et al., 2012 and references therein). In this study, temperature in combination with ammonium or alone was able to explain around 13–24% of PA and FL community variation, respectively. This is in line with previous studies, which suggest that globally temperature is the most important factor for shaping bacterial community structure in epipelagic layers (Sunagawa et al., 2015). The results of our study also indicate that a higher proportion of FL variation can be explained by environmental parameters (in this case temperature) compared to PA bacteria. This can be expected as particles, to which PA bacteria are attached, may act as a "buffer" or micro-island (Lyons et al., 2010; Yung et al., 2016), while FL cells are more directly exposed to the surrounding water. Thereby, the size of the particle is important, because the larger the particle, to which bacteria attach, the more insensitive the bacteria might be to changes in the surrounding water (Yung et al., 2016). Additionally, the high density of bacteria on particles can lead to efficient signaling and quorum sensing (Gram et al., 2002), and nutrient ratios within particles may be

very different from those in the surrounding sea water. All these factors may explain why PA bacteria are less affected by changes in environmental parameters than FL bacteria.

How Can Environmental Parameters Influence the Similarity/Dissimilarity of FL and PA Bacteria?

In this study two environmental parameters, temperature and salinity correlated with changes in Bray Curtis dissimilarities between FL and PA communities; especially when the surface upwelling samples (T1.7–T4.7; **Figure 1**) influenced by deeper water masses were excluded, and only Sur-oligo samples were taken into account. This correlation may be related to a steady temperature increase toward the equator and lower salinities due to heavy rain fall and riverine water input off Senegal. This in turn may have stimulated the genus *Oscillatoria*, which occurred at high relative abundances in the PA fraction off Senegal. *Oscillatoria* are found at intermediate salinities and are actually filamentous bacteria (Tomitani et al., 2006). While they may be PA, we suggest that they rather represented the particles themselves in this study as we observed filaments in the waters off Senegal, which were visible with the naked eye. This could even suggest that the filamentous bacteria were *Trichodesmium*, which are part of *Oscillatoria*. It has been observed previously that salinity can affect the differences between PA and FL communties, although in contrast to our results, these observations indicated higher similarities as salinity decreased (Ortega-Retuerta et al., 2013). This suggests that salinity may affect the extent to which PA and FL differ. Whether oligohaline vs. marine conditions render the two fractions more similar or dissimilar may, however, also depend on other factors.

How Can Substrate Availability Affect the Similarity/Dissimilarity of FL and PA Bacteria?

Our results show a trend, indicating that over depth, the Bray Curtis dissimilarities between FL and PA are lowest at the DCM. Although with a cruder method (CARD-FISH), focusing on larger particles, and a different depths resolution, Thiele et al. (2015) confirmed that aggregate-attached communities are most similar to the FL communities at the DCM in the same region but further offshore.

In our study both chl*a* and nutrient concentrations were elevated at the DCM. This suggests a higher substrate availability for PA and FL bacteria when compared to the oligotrophic surface stations (upwelling stations are left out because they are influenced by DCM waters) and 200 m depth stations. PA bacteria have developed different strategies to survive and thrive, and substrate colonization and resource utilization can be very complex. It has been shown that the type of particle substrate and subsequent trophic interactions during its degradation drive the succession of bacteria on particles (Datta et al., 2016). Additionally, a trade-off between colonization and dispersal of two populations of the same species of bacteria has been unraveled (Yawata et al., 2014). This indicates that PA bacteria can have different strategies to exploit the particle resources. While some are adapted to firmly attaching to a particle to thrive in this new microenvironment as best as possible, others remain flexible in order to detach and move to a new hotspot if they receive luring cues. Among others, these two publications have demonstrated the role that the available substrate plays for primary colonization and how important motility can be for some bacteria to access new particles. Under conditions with high substrate availability (as, e.g., prevalent at the DCM) moving between particle hotspots could happen more frequently and could thereby render the PA and FL community more similar. In this context the occurrence of motile PA bacteria in the FL fraction would not imply a specialization for the FL fraction, but would rather stem from their tendency to migrate between hotspots (Yawata et al., 2014).

However, also other reasons are imaginable. For example, high substrate availability at the DCM might be a cue for some PA to detach before they are exported to the deeper oceans, allowing them to stay in the surface layer. Only future studies can elucidate these hypotheses further, but as the life-styles of bacteria coincide with functional differences and therefore implications for biogeochemical cycling, it is worth investigating this topic in more detail.

CONCLUSION

This study provides the first detailed description of the BCC off the coasts of Mauritania and Senegal. As this region is not only important for global fisheries but also responsible for a large draw down of carbon, our study is vital for a better understanding of the ecosystem function in this area. The high relative abundances of several groups of Planctomycetes, Verrucomicrobia, and some Bacteroidetes (e.g., *Winogradskyella*) indicated that these microbes may represent key players in the recycling of carbon in this area. Our results can serve as a basis for future research aimed directly at microbial processes related to the export and recycling of OM in the Mauritania upwelling area.

We have demonstrated the importance of systematically distinguishing between FL and PA communities, as the two fractions can be very different in abundance and functionality. Differences between FL and PA bacteria in oligotrophic surface samples correlate with changes in salinity and temperature and the prevalent conditions off Senegal promoted the growth of *Oscillatoria*. Similarities between FL and PA bacteria were highest at the DCM and may suggest that high substrate availability reduces the dissimilarity of the two fractions. This calls for further studies on the influence of environmental parameters and substrate availability on the similarity and dissimilarity between FL and PA fractions of heterotrophic bacteria and their respective biogeochemical functions.

Our study provides a valuable basis for future studies, which should focus on seasonal changes in microbial community structure and related biogeochemical function as well as changes in the similarity/dissimilarity of FL and PA as the upwelling region expands and contracts within the Mauritania upwelling area.

DATA AVAILABILITY

The environmental dataset generated for this study can be found at PANGEA (https://doi.pangaea.de/10.1594/PANGAEA.889977) and sequencing data are stored at the ENA (accession number: PRJEB26997).

AUTHOR CONTRIBUTIONS

JB, HG, MI, and AG conceived and designed the study. JB, TH, and GK performed the field and lab work. JB, TH, and CH performed the data analyses. JB wrote the manuscript. HG, CH, MI, GK, and AG contributed to the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.02836/full#supplementary-material

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CHAPTER III

The shelf biological carbon pump off North-West Africa

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Keywords: Prokaryotes, Biological carbon pump, bacterial community composition, aggregates, shelf, Senegal, Mauritania upwelling system

ABSTRACT

Due to its high biological carbon pump (BCP) efficiency, the Canary Current is important for regulating the climate through organic carbon transfer from the oceans' surface to the deep. Off North West Africa, one of the most productive regions globally, carbon export is fuelled by perennial and seasonal upwelling occurring within the shelf region off Mauritania and Senegal, respectively. During the Meteor cruise M129, we investigated several key aspects of the BCP in the shelf area off NW Africa. We experimentally determined the free-living (FL; 0.2-3 μm), particle-attached (PA; <3 μm) and single aggregate-attached (AG; >0.3 mm) lifestyles of local bacteria to better predict their role in particulate organic matter degradation. Further, we monitored changes in bacterial communities attached to settling aggregates when subjected to different water masses, simulating lateral or vertical transport. Our results indicate a highly efficient shelf BCP, i.e. high aggregate formation potentials, microbial respiration rates and sinking velocities. Although AG bacterial community composition (BCC) was largely determined at the beginning of the experiment, we observed exchange with bacteria from the surrounding sea waters. Furthermore, when exposing aggregates to different vertical water masses, to simulate their settling through the water column, the AG bacterial communities shifted: While the fraction of Alphaproteobacteria decreased, Gammaproteobacteria and Flavobacteria increased. In contrast, simulated lateral transport did not lead to comparable shifts in BCC. Since it is known that shifts in BCC can affect carbon turnover rates, future studies should investigate, if the observed changes in BCC can also influence the BCP efficiency in the Canary Current system.

INTRODUCTION

Upwelling regions associated with Eastern Boundary Currents are among the most productive ecosystems in the world. The arrival of nutrient-rich subsurface waters at the surface stimulates primary productivity, export of organic carbon and microbial nutrient cycling (Capone and Hutchins 2013). The NW African upwelling area within the Canary Current system is one of the four major coastal upwelling systems associated with Eastern Boundary Currents (Carr 2002). Due to its strong biological carbon pump (BCP), the Canary Current system plays an important role in regulating the global climate by exporting organic carbon out of the epipelagic zone to deeper waters (Arístegui et al. 2009; Fischer et al. 2016). Productivity and carbon export off Cape Blanc, Mauritania, are fuelled by the trade-wind driven upwelling of cold and nutrient-rich subsurface water masses, which occurs throughout the whole year (Bory et al. 2001). Adjacent higher and lower latitudes, however, have seasonal upwelling patterns, linked to changes in trade winds and the migration of the Intertropical Convergence Zone (Schneider et al. 2014). South of Cape Blanc, towards Senegal, upwelling only occurs during winter (Arístegui et al. 2009). Additionally, the NW African upwelling region is influenced by Saharan dust input (Prospero 1996), whose ballasting effect has the potential to increase organic matter export from the surface to the deep sea (Iversen et al. 2010).

Primary productivity off Mauritania was calculated to be between 110 g organic C m^{-2} y^{-1} (further offshore at 21°N 31°W) and 500 g organic C m^{-2} y^{-1} (at the shelf break; (Morel 2000)). The BCP beyond the shelf break off NW Africa, especially at Cape Blanc (i.e. < 17°70'W), has received substantial interest of marine scientists: Summaries on mass fluxes and particulate organic matter (POM) transport processes off Mauritania are available by Karakaş et al. (2006) and Fischer et al. (2009b). Examples for local studies include the

investigation of POM fluxes at a mesotrophic (21°W) and oligotrophic site (31°W) offshore of Mauritania (Bory et al. 2001). Among others they found that the carbon export at the mesotrophic site, closer to the Mauritania margin, was influenced by upwelling events. Fischer et al. (2016), analysed sediment trap data from the NW African upwelling reagion to determine carbon fluxes, flux components and the variability in carbon fluxes on a seasonal to decadal timescale. They found inter-annual changes in mass fluxes and suggested that dust outbreaks enhance POM sedimentation in this area, as has been shown earlier by Iversen et al. (2010).

Additionally, several studies have measured organic matter sinking velocities, bacterial carbon turnover, microbial dynamics, and Saharan dust ballasting of aggregates collected beyond the continental shelf break (18°42'W) off Cape Blanc (Ploug et al. 2008; Thiele et al. 2015; Flintrop et al. 2018; van der Jagt et al. 2018). Iversen et al (2010) used camera profiles and sediment traps to determine size distribution and abundances of particles/ aggregates and carbon fluxes off Cape Blanc, Mauritania. They found that particles/ aggregates in this region were mostly small and fast settling due to ballasting with Saharan dust and coccoliths.

All these studies have investigated important aspects of the BCP in the NW African upwelling region. However, apart from its important role for global carbon export, marine particulate organic matter constitutes unique microcosms for marine microbes in the water column (Grossart, 2010). As microbes are involved in the degradation of marine particles, in particular of macroscopic organic aggregates (marine snow), they are important for determining the BCP efficiency. Specifically within the NW African upwelling system, knowledge about the microbial community dynamics at the microscale can help to elucidate mechanisms and hence the functioning of the oceanic BCP in more detail.

Microbiologists have seperated pelagic bacteria into free-living (FL) and particle-attached (PA) fractions (Grossart 2010). The truly FL bacteria spend their whole life-cycle suspended

in the water column, whereas truly PA bacteria generally remain associated with different kinds of particles, which in this definition includes aggregates. This contradicts another definition, where particles are seen as a single entity (e.g. a single phytoplankton cell), whereas aggregates are clusters of these single entities (e.g. marine snow or fecal pellets), which are often stuck together by a glue (e.g. transparent exopolymer particles; TEP) (Riley 1963; Alldredge et al. 1993; Passow 2002). Once an aggregate exceeds a size of 0.5 mm, it is defined as marine snow. Therefore, depending on the type of definition, particles may either include aggregates or individual entities.

In this article we use the following definitions:

1. Aggregate-attached (AG) bacteria: Bacteria, attached to single macro-aggregates (>0.5 mm), which were picked and sequenced individually

2. Particle-attached (PA) bacteria: Bacteria, associated with particulate organic matter, such as "true" particles and clusters of particles (aggregates). They can even include large filamentous bacteria (Bachmann et al. 2018). In our study they were captured on a filter with 3.0 µm pore size (e.g., Kegler et al. 2017; Bachmann et al. 2018).

3. Free-living (FL) bacteria: Bacteria, which are typically collected on filters with a pore size of 0.2 µm after the removal of the PA bacteria

Laboratory studies investigating microbial dynamics on marine particles were, e.g., carried out using model particles made of chitin or agar spheres (Kiørboe et al. 2003; Datta et al. 2016). Datta et al. (2016) observed clear temporal successions during which prevalence of primary degraders over time shifted towards a secondary consumer dominated community. Kiørboe et al. (2003) found that PA bacterial communities were grazer controlled and that there was a continuous exchange between the FL and PA populations. Modelling studies suggest that bacteria (and eukaryotic microbes) rapidly colonize fresh particles/ aggregates

and abundances reach a steady state after about 0.5 days (Kiorboe 2003). This indicates that initial colonization is important for determining the PA bacterial community composition (BCC) (Bižic-Ionescu et al. 2018). Off Mauritania only few studies have investigated the microbial community composition on particles/ aggregates so far (Thiele et al. 2015; Bachmann et al. 2018). Thiele et al. (2015) suggested that the PA community at depth originates from the BCC in the upper mixed layer and that processes within individual particles/ aggregates, such as grazing and substrate competition, determines the deep BCC more than attachment/ detachment dynamics at depth. Bachmann et al. (2018) suggested that vertical substrate availability is highest at the DCM and, thus, determines similarities/ dissimilarities between FL and PA bacteria at this depth by stimulating attachment/ detachment of PA bacteria.

The PA and AG BCC has the potential to impact carbon degradation and thereby the efficiency of the BCP (Enke et al. 2018). However, particles and aggregates are not only exported vertically. Off Mauritania, filaments and Eddies contribute to the offshore transport of organic carbon (Arístegui et al. 2004). Therefore, during Meteor cruise M129 to the shelf area of Mauritania and Senegal, we will investigate PA and AG BCC dynamics and their potential exchange with FL bacteria. Using flow through roller tanks (FTRT), we will simulate the vertical and horizontal transportation of particles and aggregates.

We hypothesize that the shelf area off Mauritania and Senegal will be characterized by high aggregation volumes and sinking velocities, fueling the BCP in this area. During vertical export, PA/ AG microbial respirations rates and BCC are expected to affect the efficiency of the local BCP. During horizontal export, PA/ AG microbial dynamics have the potential to contribute to remineralized productivity. We expected that PA and AG BCC are primarily determined by the initial bacterial colonization, but subjection to different surrounding water may have the potential to change BCC and thus their function.

MATERIALS AND METHODS

The Meteor M129 cruise started from the Azores, on July 30th and finished on the Cape Verdes on August 25th 2016 (Figure 1). Four rolling tank experiments, (E-oligo, E-eddy, E-upw, E-Sen) were carried out during the cruise: E-oligo (36.07917;-25.04111) started in the oligotrophic offshore waters, E-eddy (26.83028, -26.33806) was initiated with water from an eddy, E-upw (19.90639, -17.01944) started in the core of the perennial Mauritania upwelling and E-Sen (13.68222, -17.44583) off the Senegal coast. The respective experimental procedures are shown in Figure 1 and can be summarized as followed:

Twenty conventional **r**oller **t**anks (RT) and two closed (i.e. no flow at the beginning) **f**low **t**hrough **r**oller **t**anks (FTRT) were used to form aggregates (AG, marine snow). Nutrients (NUT), dissolved organic carbon (DOC), particulate organic matter (POM), transparent exopolymer particles (TEP), FL, and PA and AG bacterial communities were determined in triplicates (s. Figure 1). However, no large single aggregates (marine snow) were present at the start of the experiment (T_0) and were only sampled from T_1 onwards. The time points sampled were T_0 (start of incubation), T_1 (FTRT were opened and fresh sea water was introduced into the system) and T_2 (end of incubation), s. Table 1. Two types of flow waters were introduced into the FTRT at T_1: Surface (Flow) water (E-oligo and E-upw) and 100/ 200 m deep reservoir (RES) water (E-eddy and E-Sen). While Flow water could be directly taken from the ship's membrane pump, the RES water was taken using a CTD and then stored in darkness at 10°C in large tanks of ca. 60 L for logistical reasons. Experiments with Flow water (E-oligo and E-upw) were carried out in a constant climate room (ca. 22°C +/- 1°C) while the FTRT of E-Sen and E-eddy were moved to the 10°C room at T_1. Unfortunately, FL, PA and AG BCC are only available for the last three experiments, i.e. E-eddy, E-upw and E-Sen.

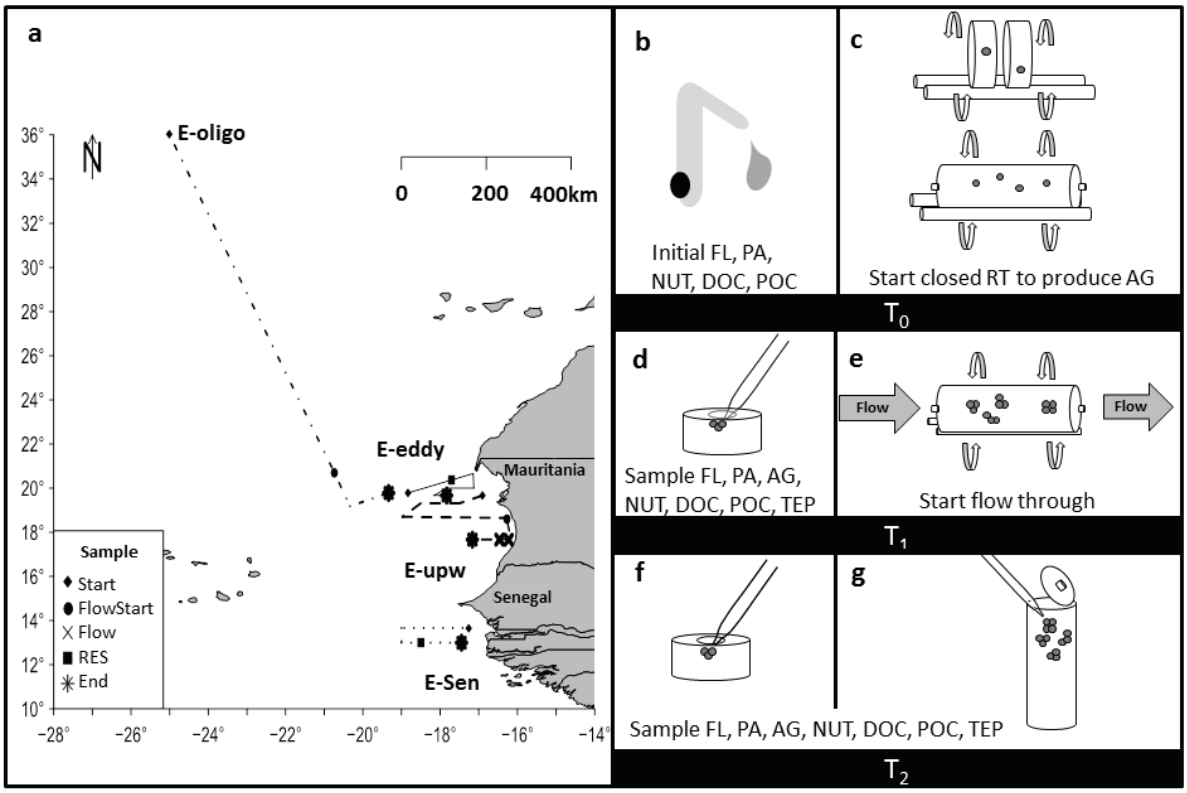

Figure 1. Cruise track of M129 and locations of the four experiments (a) E-oligo, E-eddy, E-upwelling (E-upw) and E-Senegal (E-Sen). Dotted line along the coast represents the 100 m depth isobath and thus the edge of the shelf region. At T_0 (start) roller tanks (RT) and flow through roller tanks (FTRT) were filled with membrane pump water (b) of the indicated location (see a). Flow through and closed (i.e. no flow) roller tanks were placed onto the roller tables and started rolling to form marine snow (c). At T_1 FL, PA and AG were sampled from the roller tanks (d) and the FTRT were opened ("FlowStart"; e). Surface (Flow) or 100/ 200 m deep water (RES) was introduced into the FTRT. The Flow water was sampled from the membrane pump at indicated locations (see a) in E-upw, whereas the reservoir (RES) water was taken using a CTD at the indicated locations (a) and then stored in large tanks of ca. 60 L. At T_2 (the end of each experiment) both FTRT and RT were sampled before rinsing the tanks thoroughly with Milli-Q water. Abbreviations: AG= aggregate-attached communities, FL= free-living bacterial communities, PA= particle-attached communities, DOC= Dissolved organic carbon, POC= particulate organic carbon, TEP= transparent exopolymer particles

Rolling tank experiments

Due to turbulence and the constant rotation of sea water against gravity, aggregate formation is facilitated in conventional, closed rolling tanks (Shanks and Edmondson 1989). In replicate (n >10) 1.1 L Plexiglas cylindrical tanks, aggregates were formed at constant temperature (ca. 22°C +/-1°C), in darkness and 3 rpm for up to 24 h (especially in oligotrophic regions these longer periods of incubation were needed). Depending on the amount of aggregates that was available for sampling at T_1, different numbers of closed roller tanks were left undisturbed and kept for sampling at T_2. This resulted in ten (E-oligo), five (E-eddy), thirteen (E-upw) and three (E-Sen) closed RT at T_2 for the respective measurements. After the formation of visible aggregates at T_1 (Table 1), they were picked for further analysis.

In order to circumvent the bottle effect, as well as to study the attachment and detachment of bacteria to and from aggregates, recently, a new method, the FTRT, has been developed, (Ionescu et al. 2015). This system allows the sinking of aggregates through a medium that can be renewed continuously. In our study, two FTRT were filled and closed at T_0 to allow the formation of aggregates just as in the RT. We had to close the FTRT at the beginning as our initial tests revealed that smaller particles were washed out hindering aggregate formation in FTRT, which remained in "flow through mode" from the beginning. At T_1, after an initial phase of aggregate formation, the FTRT were operated in flow through mode with 125 µm pre-filtered *in situ* collected sea water. Flow rates were adjusted to 3-10 mL per minute, which is close to what Ionescu et al. (2015) suggested, in order to balance the renewal of water and the fragility of aggregates, which may break apart at higher flow rates. During E-oligo and E-upw surface (Flow) water was introduced to the FTRT to mimic lateral transfer of aggregates along the continental shelf area. Therefore, the FTRT was connected to the membrane pump of the ship. For E-eddy and E-Sen 100 m and 200 m reservoir (RES) water was introduced to the FTRT after T_1 to mimic vertical sinking of the aggregates in the continental shelf region. RES water was taken using a SBE32 Rosette water sampler with 24 times 10 L Niskin bottles

(s/ n 0342) and stored in large containers for a maximum of 3d until the end of the experiments. In order to subject a higher number of aggregates to the Flow/ RES waters, selected aggregates were picked from closed roller tanks using a clean plastic bore pipette at T_1 and placed into the two FTRT. At T_2, aggregates were carefully sampled from the FTRT using sterile glass pipettes with cut off tips.

Table 1. Description of the four experiments carried out on board of the RV Meteor (M129 cruise). T_0 represents the start of the experiments, T_1 is the first sampling time during which the conventional roller tanks were sampled and selected aggregates were transferred into the flow through rolling tanks. At T_2, the second and last sampling time point of the experiments, all remaining closed and the flow through rolling tanks were sampled.

EXP	T_0Date[1]	T_0:T_1[2]	T_0:T_2[3]	T_0:V_1[4]	T_0:V_2[5]	T_0:V_3[6]	T_0Water[7]	EXPwater[8]
E-oligo	30.07.2016	98	124	24	48	NA	0.1	Flow 837-840
E-eddy	05.08.2016	36	85	20	34	67	840	RES 842
E-upw	10.08.2016	51	102	24	43	NA	878	Flow 889-892
E-Sen	18.08.2016	43	88	23	NA	NA	908	RES 919

[1] Start of the experiments

[2] Time in hours from the start of the experiment until T_1

[3] Time in hours from the start of the experiment until T_2

[4] Time in hours from the start of the experiment until first video

[5] Time in hours from the start of the experiment until second video

[6] Time in hours from the start of the experiment until third video

[7] Start water for the experiments refer to Meteor cruise stations (https://www.pangaea.de/ddi?retr=events/Meteor_1986/M129.retr&conf=events/CruiseReportHTML.conf&title=Station+list+of+cruise+M129&format=html); Note for E-oligo there was no official M129 station but the sample location can be seen in Figure 1.

[8] Station names, at which the water, which circulated through the FTRTs from T_1 until T_2, was collected

Chemical sea water parameters

Particulate organic carbon (POC) samples were taken at all three time points (T_0, T_1 and T_2) of the experiment from different roller tanks and at indicated time points when adding the RES water (Table 1). Between 1000-5000 mL of marine water were filtered through pre-combusted (450°C for 4 h) and pre-weighted 47 mm GF/F filters (Whatman, Dassel, Germany). Filters were dried at 40°C for at least 24 h and total carbon (TC) and total nitrogen (TN) concentrations were measured on an Elemental Analyzer (EA-3000, EuroVector, Italy). The organic carbon fraction (POC) was measured after acidification of the filter with 1N HCl to remove the inorganic carbon. CN ratios were calculated from POC and TN.

Dissolved inorganic nutrient (NUT) samples were taken in triplicates from the POC filtrate. Using a continuous segmented flow auto-analyser (San System, Skalar, the Netherlands) the concentrations of five NUT, i.e. nitrate, nitrite, ammonium, phosphate and silicate, were determined according to Grasshoff et al. (1999).

Triplicates of dissolved organic carbon (DOC) concentrations were also obtained from the POC filtrate and preserved frozen at -20°C. Acidified DOC samples were analysed using the high-temperature combustion method (Dafner and Wangersky 2002) with a TOC-VCPH TOC autoanalyzer (Shimadzu, Mandel, Canada).

TEP were quantified according to Engel (2009), which is based on the spectrophotometric method introduced by Passow and Alldredge (1995). For the calibration, this method relates the absorption of alcian blue (a dye staining TEP) to a standard curve of known masses of gum xanthan (GX), which is used as a TEP equivalent. Then triplicate environmental TEP samples are stained with alcian blue and their absorption is measured and compared to the standard curve. We followed the standard protocol with two modifications: For the calibration, typically a GX (undefined mesh size) solution with a known concentration is prepared and a known volume is filtered on polycarbonate filters. However, since the GX

does not always dissolve homogeneously, the filters are weighted and the mass of GX is used for the calibration. In this study we used GX with a mesh size of 200, which appeared to dissolve homogeneously, when inspected by eye. Since weighting of the small masses of GX, as usually applied for this method, can produce bias affecting the calibration curves, we omitted this step and calculated the expected mass of GX based on the known volume of GX instead. An example for the resulting calibration curve is found in the appendix (Supplementary Figure S1).

Video determination of aggregate sizes, sinking velocities and aggregation volume

The area of aggregates and their respective sinking velocities in closed rolling tanks were analyzed by video according to Ploug et al. (2010) at V_1, V_2 and V_3 (if applicable; Table 1). The potential of sea water to form aggregates over time was determined from the collected video material. Aggregate area was converted into equivalent spherical diameter (ESD), from which the total aggregated volume (assuming spherical shape as RT aggregates tend to be more spherical) was calculated at V_1 and V_2.

Flow chamber measurements to determine the size, sinking velocities and O_2 fluxes of selected aggregates

Five randomly selected aggregates were chosen for O_2 consumption measurements. They were picked at T_1 from RT using plastic bore pipettes and placed into the flow chamber. The dimensions of each aggregate were measured along two axes (length and height) using a calibrated horizontal microscope. The equivalent spherical diameter (ESD), volume and surface area were calculated assuming spherical shape. The sinking velocity of each aggregate was measured in triplicates by adjusting the upward flow within the flow cell until the aggregate was floating one diameter above the mesh (Ploug and Jørgensen 1999; Ploug et al. 2010). Oxygen measurements were performed according to Ploug et al. (1999, 2008). Therefore, oxygen profiles from above the aggregate, through the boundary layer to the centre of the aggregate were measured with a 25 µm step size using a microelectrode with a tip

diameter of 10 µm (Revsbech 1989). A diffusion-reaction model to calculate the oxygen uptake rates and to estimate the apparent diffusivity inside aggregates was applied to measure the O_2 consumption rates in each aggregate (Ploug et al. 1997). Carbon respiration rates were calculated from oxygen consumption rates by assuming a respiratory quotient of 1 mol O_2 to 1 mol CO_2 (Ploug and Grossart 2000).

Organic carbon content of aggregates

At T_1 of E-eddy (n= 2) and E-Sen (n= 1) known amounts of small aggregates (ESD ca. 0.5-3 mm) were filtered onto pre-weighed 25 mm GF/F filters (Whatman, Dassel, Germany). The filters were rinsed with de-ionized water and dried at 40°C (>48 h) before being re-weighed on a Mettler Toledo balance (sensitivity: 0.1 mg). The organic carbon (POC) fraction was measured using an Elemental Analyzer (EA-3000, EuroVector, Italy) after acidification of the filter with 1N HCl to remove the inorganic carbon. For calculations of carbon specific respiration rates the average of the three measurements were used.

Scanning electron microscopy (SEM) imaging

Selected aggregates from each experiment (E-oligo: n= 3, E-eddy: n= 1, E-upw: n= 4 and E-Sen: n= 2) were frozen for SEM and energy- dispersive x-ray spectroscopy (EDX). For analysis, the samples were thawed and carefully rinsed with deionized water by filtration using a 0.22 µm polycarbonate filter in order to remove all salts. Filters were placed onto a stub with a carbon mounting tab and dried in the oven for 2 h at 35°C. Dry filters were sputtered with gold under high vacuum for 30 s using a Cressington Sputter coater 108auto/SE. Overview photos were taken of each aggregate before investigating it in more detail. EDX analysis, using the software AZtecEnergy (OXFORD), was carried out on selected structures of interest within the aggregates to understand their chemical composition.

FL, PA and AG microbial community composition

AG bacteria were sampled by picking all visible aggregates from rolling tanks and transferring them into separate sterile Eppendorf tubes for storage at -20°C until DNA extraction. Free-living (FL) and particle-attached (PA) bacteria of all sea water, i.e. Start, Flow, RES, RT and FTRT, used in the experiments were separated via sequential filtration through 3.0 µm and 0.2 µm Nucleopore TrackEtch polycarbonate membranes (Whatman, Dassel, Germany), respectively (Kegler et al., 2017).

DNA extraction was carried out after Nercessian et al. (2005) but skipping a second chloroform washing treatment. LGC genomics (Berlin, Germany) carried out amplicon sequencing of FL, PA and AG BCC using the primer pair Bakt_341F (5'-TCCTACGGGGGCWGCAG-3') and Bakt_805R (5'-TGACTACHVGGGTATCTAAKCC-3'). These primers target the hypervariable regions V3-V4 of the bacterial 16S rRNA gene (Klindworth et al. 2013). Sequencing was performed on an Illumina MiSeq using V3 Chemistry (Illumina) in a 2x 300 base pair paired-end run.

Bioinformatic analyses

The company LGC genomics carried out demultiplexing and the removal of primer sequences from the raw paired-end reads with *cutadapt* (Martin 2011). Sequences were further analysed according to Hassenrück et al. (2016). In summary, sequences were quality trimmed (sliding widow of 4 bases and an average quality of 15) with *trimmomatic* v.033 (Bolger et al. 2014). *PEAR* v0.9.6 (Zhang et al. 2014) was used to merge sequences and *swarm* v2.1.1 was applied to cluster operational taxonomic units (OTU; Mahé et al. 2014). Taxonomic classification with *SINA* v1.2.11 was carried out using the SILVA rRNA project reference database release 128 (Pruesse et al. 2012). Singletons, doubletons, chloroplasts, mitochondria and OTUs, which were unclassified on phylum level, were excluded.

Statistical analyses

All statistical analyses were run in R studio using the core distribution (http://www.rstudio.com) and additional packages, such as *vegan* (Oksanen et al. 2015).

Sample ordinations and chemical parameters were displayed in a principal component analysis (PCA). Parameters with missing values (TEP, POC and N) were added to the PCA using *envfit*.

Differences in aggregation volume were tested using a Kruskal Wallis test because of the non-normal distribution of the data. A post hoc (Dunn's test, (Dunn 1964)) was used to identify, which samples differed in their aggregation volume. The level of significance, α, was set to 0.05.

Differences between FL, PA and AG BCC were calculated as Bray Curtis dissimilarities and used for cluster analysis (unweighted pair-group method using arithmetic average, UPGMA). Ordination of FL, PA and AG BCC was carried out using non-metric multidimensional scaling (NMDS) on relative sequence abundances of FL, PA and AG OTUs.

Chemical sea water parameters and sequencing data will be made available on PANGAEA and ENA, respectively (accession numbers pending).

RESULTS

Sea water parameters

The chemical sea water parameters were ordinated in a principle component analysis (PCA; Figure 2) and raw values are displayed in the Supplementary Table S1. In the PCA, the first two principal components (PC1 and PC2) explained 72% of the variability in the data set. PC1, which explained 56% of the variability, was mainly influenced by inorganic nutrients (especially silicate), which were positively correlated with PC1 and mainly seperated the deep RES samples from surface samples. PC2 was negatively influenced by organic matter, such as DOC, N and POC.

As the experiment E-oligo was carried out in oligotrophic offshore waters its Start and Flow waters were both characterized by low inorganic nutrient concentrations (e.g. NOx= 0 µM, PO_4= 0.09-0.15 µM and SiO_4= 0.75-1.14 µM).

E-eddy also started in oligotrophic waters with similar inorganic nutrient concentrations but with POC concentrations of ca. 450 µg L^{-1}. Apart from DOC concentrations, which increased from around 500 µM at T_0 to around 850 µM at T_2, the closed systems experienced similar inorganic nutrient and organic matter concentrations from the beginning until the end of the experiment. The RES waters taken from 100 m at station 1.6 contained high inorganic nutrient concentrations, such as NOx (ca. 25 µM) and PO_4 (1.1-1.6 µM). The two FTRT contained waters that were very similar to the RES water at T_2. This indicates that the oligotrophic surface waters had been completely replaced by the nutrient-rich RES waters and that FL, PA and AG bacteria in the RT vs FTRT experienced very different conditions after T_1.

E-upw started within the upwelling waters off Mauritania and was the only experiment where NO_3 concentrations (7.53 µM) were above 0 µM at T_0. Other inorganic nutrients, such as PO_4

(1 µM), NH_4 (0.68 µM) and SiO_4 (4.53 µM), were also present in higher concentrations at the start of E-upw. At T_1, inorganic nutrient concentrations had decreased by about half of their initial concentrations. FTRT were subjected to surface Flow water originating from outside the seasonal upwelling area.

Elevated DOC concentrations of around 547 µM as well as low inorganic nutrient concentrations at the start of E-Sen led to its ordination close to the DOC arrow within the PCA. In RT at T_2, all inorganic nutrients were nearly depleted and DOC concentrations were around 197 µM. As 200 m water was introduced to the FTRT after T_1, the RES water as well as the water inside the FTRT had increased inorganic nutrient concentrations (NOx= 22 µM, PO_4= 1.05 µM and SiO_4= 7.7 µM).

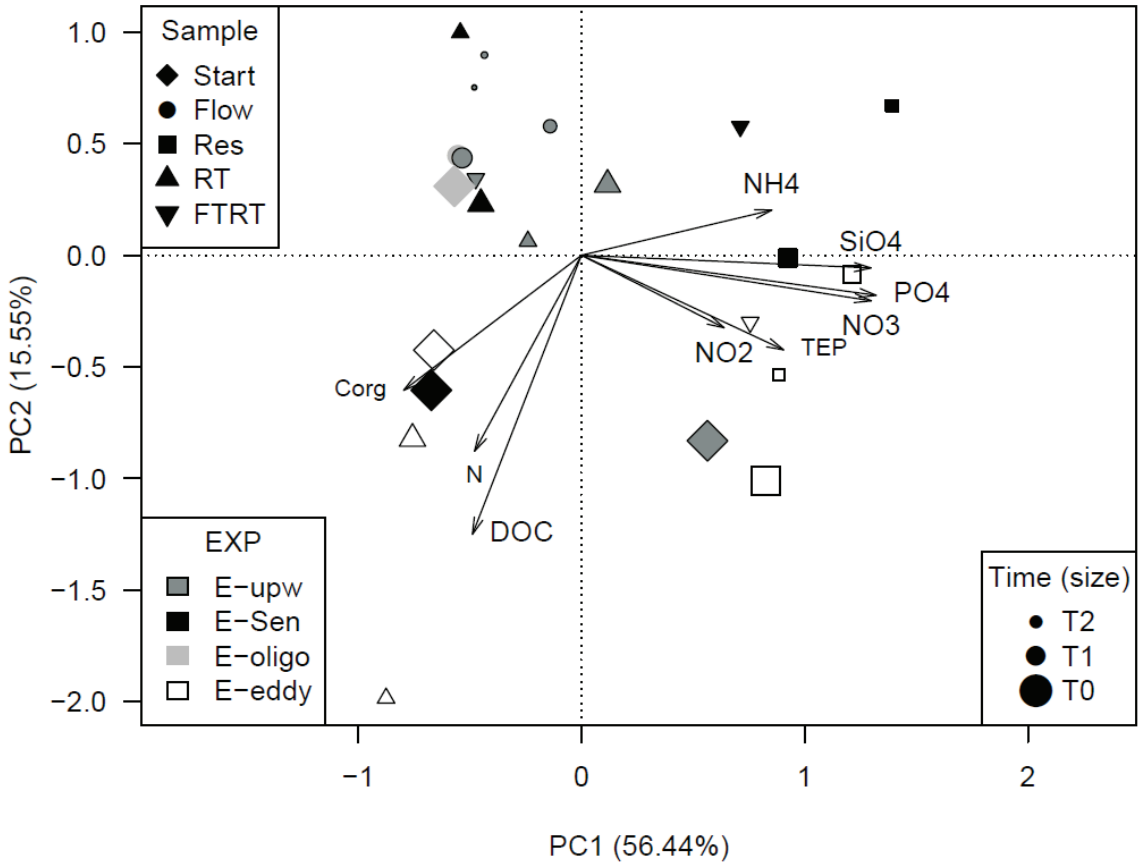

Figure 2. Principal component analysis (PCA) of organic matter and inorganic nutrients measured during the four experiments. The size of each point indicates its sampling time (T_0, T_1 or T_2). Due to missing values in some samples, particulate organic carbon (POC/ C_{org}), particulate organic nitrogen (N) and transparent exopolymer particles (TEP) were added to the PCA using *envfit*.

Aggregation volume

The aggregation volume (Figure 3) slightly differed between the four experiments in the first video (V_1; after around 20-24 h of incubation; see Table 1). While E-oligo had a mean aggregation volume of around 0.5 mm^3 L^{-1}, the E-eddy, E-upw and E-Sen had mean aggregation volumes of 1.1 mm^3 L^{-1}, 3.13 mm^3 L^{-1} and 1.8 mm^3 L^{-1}, respectively. However, in E-upw the heterogeneity of the recorded aggregate volumes was very high. A Kruskal Wallis test indicated significant differences ($p < 0.001$) between the aggregation volumes at T_1 of the four experiments. A post hoc (Dunns) test suggested that the aggregation volumes of E-oligo and E- upwelling as well as E-oligo ($p= 0.0008$) and E-Sen ($p= 0.009$) at T_1 were significanlty

diffferent. In the second video (V_2; 48, 34 and 43 h after the start of the experiments E-oligo, E-eddy and E-upw, respectively), the aggregation volume of E-upw increased to the highest observed mean (around 22.37 $mm^3 L^{-1}$) in this experiment. The aggregation volume of E-oligo increased to 1.74 $mm^3 L^{-1}$, while E-eddy did not change much (1.3 $mm^3 L^{-1}$), which probably resulted from its shorter incubation time (34h for E-eddy vs >40 h for E-oligo and E_upw) between V_1 and V_2.

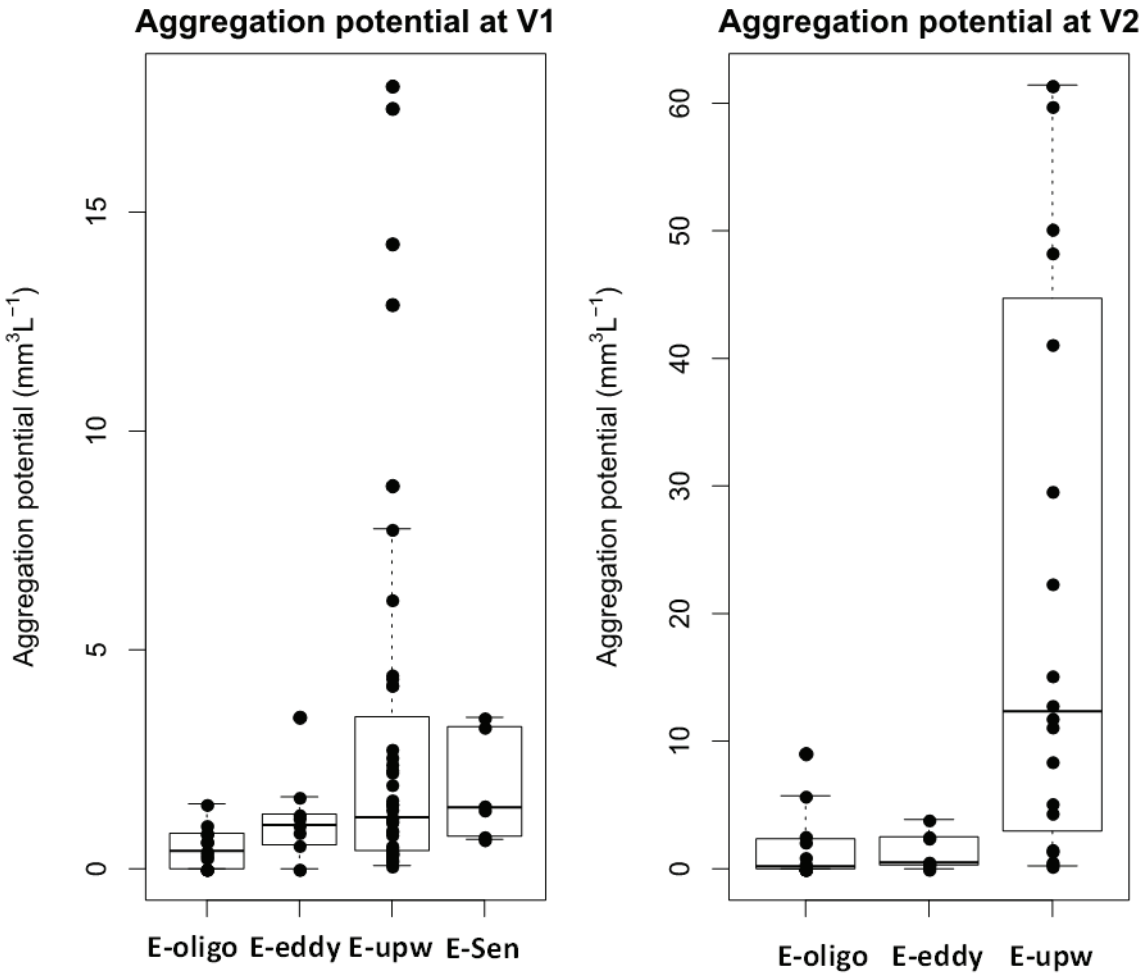

Figure 3. Aggregation volumes of the four experiments at V_1 (20-24 h after T_0) and V_2 (34-48 h after T_0).

Composition of aggregates

Using scanning electron microscopy (SEM) most of the main phytoplankton phyla (i.e. diatoms, coccolithophores, foraminifera and radiolarian) were found in aggregates of all

experiments (Supplementary Figure S2). Apart from those of E-oligo, flagellates occurred on all aggregates. Structures of different genera of coccolithophores (Supplementary Figure S2 K) were present and abundant within many of the aggregates. The SEM images of selected aggregates indicated that the overall composition of aggregates formed during the three experiments differed substantially: While those aggregates originating from E-oligo and E-eddy were dominated by rod and coccoid shaped structures rich in calcium and oxygen (A-D), aggregates from the other two experiments were characterized by a higher diversity of diatoms and coccolithophores.

Sinking velocities of aggregates

Sinking velocities of aggregates in the roller tanks varied between -100 (i.e. floating) to 370 m d^{-1} and there was no correlation (r= 0.002) between size and sinking velocity when experiments were analysed together (Figure 4). Most replicate measurements (n= 18 at V_1 and n= 16 at V_2) were possible for E-upw, where a trend towards bigger and fast sinking aggregates could be observed with time. Highest sinking velocities of up to 370 m d^{-1} were observed in E-Sen (n= 5). In E-eddy, sinking velocities of the generally smaller aggregates (n= 6) at V_1 were mostly above 200 m d^{-1}, whereas the larger aggregates (n= 4) at V_2 had slower sinking velocities of only around 15 m d^{-1}.

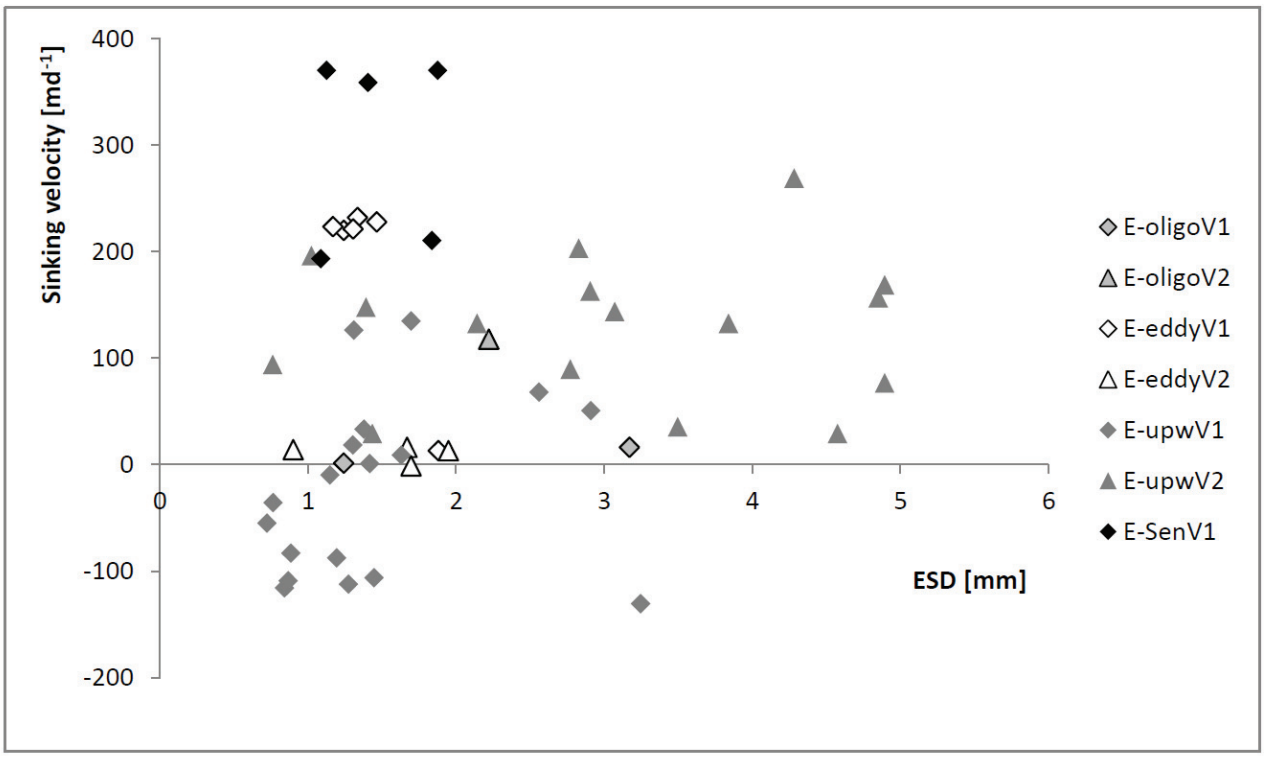

Figure 4. Sinking velocities of aggregates in rolling tanks of all four experiments at V_1 and V_2.

Carbon specific respiration rates of aggregates

Carbon specific respiration rates (Table 2) were between 0.23 and 0.76 d^{-1} for all of the measured aggreates with one exception: One of the aggregates formed in E-Sen, which had incoprorated a dead amphipod, was anoxic in its centre.

Table 2. Results of microsensor measurements on microbial carbon specific respiration rates in aggregates.

AG	Exp	ESD mm^2	Sink Vel md^{-1}	Rep	Average Flux $nmolO_2cm^{-2}h^{-1}$	Sur area AG cm^2	Integrated Fux $nmolO_2agg^{-1}d^{-1}$	C content $\mu g\ agg^{-1}$	R rate $\mu g\ agg^{-1}d^{-1}$	CSR d^{-1}	CSR %
1	E-eddy	0.53	NA	2	128.26	0.07	215.47	3.41	2.59	0.76	75.82
2	E-upw	1.27	28.48	3	33.88	0.21	169.93	3.41	2.04	0.60	59.80
3	E-Sen	0.79	74.56	1	39.40	0.08	78.08	3.41	0.94	0.27	27.48
4	E-Sen	0.63	44.55	2	52.67	0.05	65.02	3.41	0.78	0.23	22.88

Abbreviations: AG= number of aggregate, Exp= experiment, ESD= equivalent spherical diameter, Sink Vel= sinking velocity of aggregate, Rep are the number of replicate measurements of the same aggregate, which led to the average flux, Sur area AG= Surface area of aggregate, Integrated flux= flux integrated over surface area of aggregate, C content= POC content of aggregate, R rate= respiration rate and CSR= carbon specific respiration rate

Microbial community composition

FL, PA and AG bacterial communities are available for E-eddy, E-upw and E-Sen (Figure 5).

The start BCC of three experiments differed greatly and BCC changed further over the course of all experiments. Differences between natural (Flow/RES) communities and roller tank communities were most pronounced (Figure S3)

BCCs of FL, PA and AG fractions differed between RT and FTRT and the introduction of Flow water led to changes in the BCC. Despite major differences in the start and Flow/ RES waters of the three experiments, the RT and FTRT communities were dominated by similar bacterial families, although often with different relative sequence abundances.

The introduction of surface Flow waters into the FTRT in E-eddy led to changes in the BCC. For example, the input of Flow water re-introduced Actinobacteria in higher relative sequence abundances to the FL communities. Even the AG communities of E-upw changed and higher relative abundances of Gammaproteobacteria were observed in the FTRT when compared to the AG communities in the conventional RT.

The introduction of 100/ 200 m RES water also changed the BCC. RES waters of E-eddy and E-Sen were rich in bacterial families when they were sampled but storage in the large containers led to a bottle effect, and Gammaproteobacteria dominated the RES waters at the end of the experiments. Despite high relative abundances of Planctomycetes in the RES waters, they were (almost) absent in the PA and AG fractions of E-eddy and E-Sen. In E-eddy the introduction of RES water led to higher relative abundances of Bacteroidetes and Gammaproteobacteria in the AG fraction. In E-Sen relative abundances of Clostridia declined in the AG fraction.

Despite both PA and AG BCC being attached to particulate organic matter (although of different sizes) they differed in all experiments. In E-upw, for example, after surface waters

were introduced to the system, there were slightly higher relative sequence abundances of Alphaproteobacteria in the PA fraction than in the AG fraction. In contrast in E-eddy and E-Sen there were higher relative sequence abundances of Gammaproteobacteria in the PA than in the AG fraction at both T_1 and T_2. Furthermore, the AG fractions contained Clostridia, which were absent or only present in low relative abundances in the PA fraction.

The majority of AG communities showed a similar trend. However, aggregates can be very different (as SEM pictures also confirmed), leading to heterogeneity in the attached BCC between individual aggregates. For example, one aggregate at T_1 of E-Sen was characterized by high relative abundances of Desulfovibionaceae (a family of sulfate reducing bacteria), which were not present in any of the other aggregates. Clostridiaceae_1 were present in high relative abundances on three of the aggregates at T_1 of E-Sen, but were missing or in low relative abundance on the fourth aggregate.

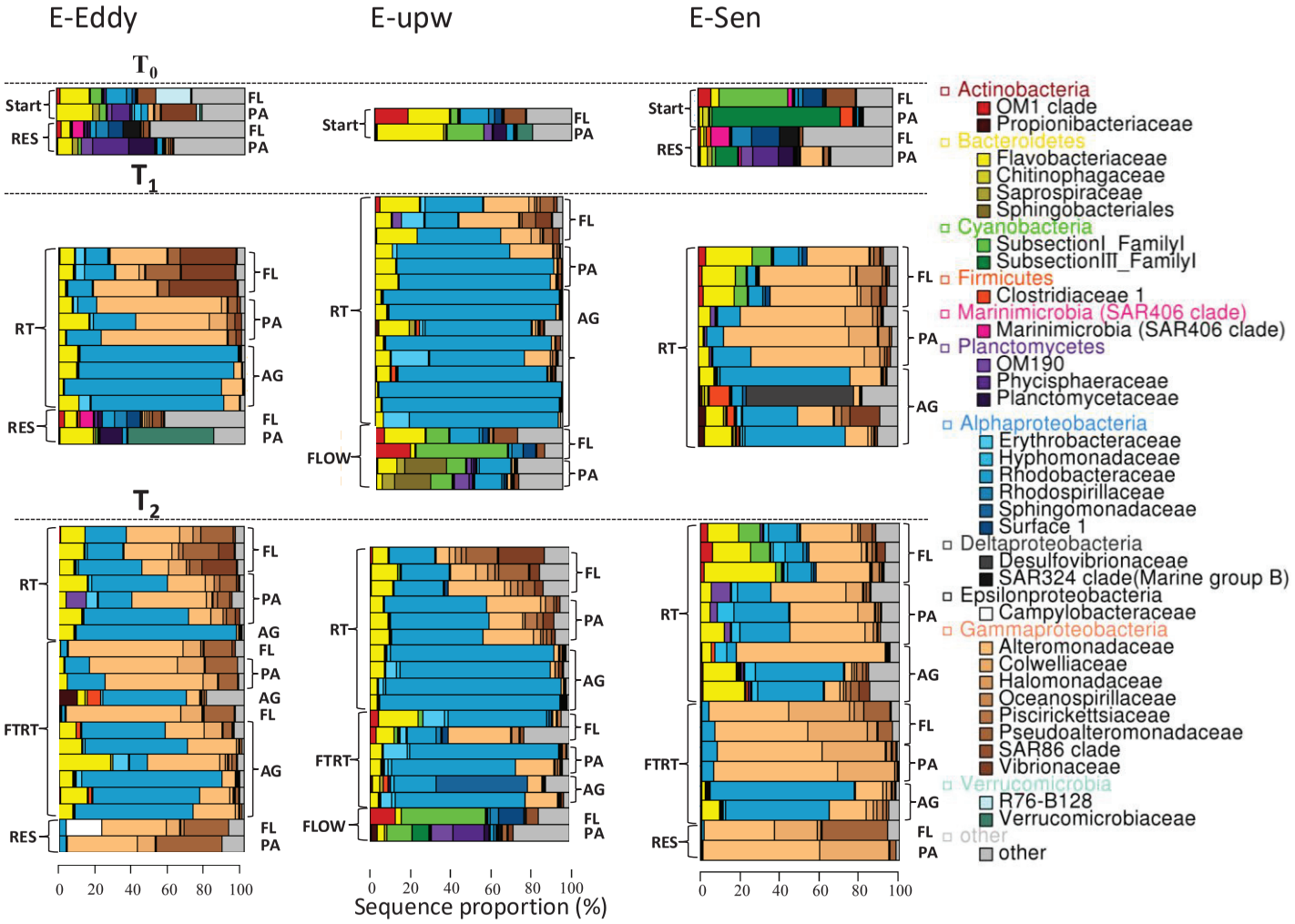

Figure 5. Sequence proportions of the most abundant free-living (FL), particle-attached (PA) and aggregate-attached (AG) bacterial families found in three experiments.

DISCUSSION

Although continental shelf regions only represent a minor fraction (around 0.5%) of the ocean's volume, they play an important role in the cycling of organic matter and inorganic nutrients (Takahashi et al. 2009). Whether or not the so called "Continental shelf pump" globally accounts for net uptake of CO_2, as suggested by Tsunogai et al. (1999) is still under debate. Off Mauritania, where upwelling and Saharan dust input lead to increased primary productivity (Bory et al. 2001), previous studies have shown a coupling between shelf and open-ocean waters due to strong, seasonal North East trade winds (Bory et al. 2001; Helmke et al. 2005).

Highest aggregation volume and sinking velocities in E-upw

We found significantly higher aggregation volumes within the shelf regions off Mauritania and Senegal compared to the oligotrophic offshore waters. This indicates that the shelf region importantly contributes to the BCP in the area.

Sinking velocities of aggregates were within the range (although slightly lower) of previously published velocities in the area (Fischer et al. 2009a; Iversen et al. 2010). They indicate a strong organic carbon export. With maximum sinking velocities of 400 m d^{-1} aggregates have the potential to reach the seafloor within a day in the shelf area (<100 m). SEM imaging revealed the presence of mineral ballast (e.g. diatom shells, coccoliths, foraminifera houses) inside aggregates, which may be responsible for the sometimes high sinking velocities, as previously shown (van der Jagt et al. 2018). Especially the composition of aggregates from E-upw appeared to be different from the aggregates of all other experiments when analyzed via SEM and indicated high ballasting via diatom shells. This may explain why their sinking velocities tended to be higher compared to the other experiments. Additionally, the proximity to the shore, which may have led to higher Saharan dust input compared to regions further

offshore, may have increased aggregate sizes and hence sinking velocities at E-upw (van der Jagt et al. 2018).

Aggregate- and particle-attached microbial degradation of POM

The life-styles of marine bacteria are linked to metabolic differences and attached bacteria generally seem to have a higher ability to re-mineralize polymeric organic carbon (Lyons and Dobbs 2012). Aggregate- and particle-attached bacteria typically possess carbon specific respiration rates around 0.1-0.2 d^{-1} (Ploug et al., 1999). Hence their activity has the potential to control the efficiency of the BCP.

Although POC concentrations of individual aggregates were within the range of previously published data for the area (Iversen et al. 2010), they were at the lower end of this range. This may explain the elevated carbon specific respiration rates observed in this study when compared to previous results of the region and should thus be treated with caution (Iversen et al. 2010).

Flow through rolling tanks: Improvements for future studies

As it is difficult to follow individual aggregates on their horizontal or vertical journey through the water column *in situ*, we applied FTRT, which were designed to experimentally study PA bacteria but minimizing the bottle effect (Ionescu et al. 2015). By flushing the tanks with surface or mesopelagic waters, we wanted to track the effect of different water masses on previously formed aggregates. As this was the first application of the FTRT in the marine environment, we would like to share our experience in order to improve future applications of this useful tool. Unfortunately, we had to form all aggregates in closed RT and FTRT (from T_0 until T_1), which resulted in an obvious bottle effect, even to some extent in the FTRT. In the test phases prior to the cruise, we experienced a reduced aggregate formation when the FTRT remained opened from the beginning on, probably because smaller particles were easily washed out of the system. Therefore, if FTRT are used in the future with the intention

of avoiding the bottle effect, we suggest a shorter initial incubation time and/ or flow through with a very low flow rate to still allow for organic matter aggregation.

Despite the unfortunate development of a bottle effect, the aim to track changes in PA and AG communities, when these particles/ aggregates were subjected to epi- or mesopelagic flow water was still achieved.

Bacterial attachment to precolonized aggregates

In this study we observed that the majority of the AG BCC was determined at the start of the experiment. This is in line with previous studies, which have shown that PA and AG bacteria at all depth mainly originate from the photic zone (Thiele et al. 2015; Pelve et al. 2017; Mestre et al. 2018). Continuous fresh sea water input in the flow through systems, however, resulted in sometimes pronounced changes in BCC. While this was expected for the FL and possibly also the PA fraction, which may be replaced by the Flow/ RES water communities due to flushing, changes in the AG fraction indicated attachment and also detachment of bacteria to and from the large aggregates. Around 20-80% of marine bacteria are motile and and chemotactic motility can guide them to nutrient and OM patches (Grossart et al. 2001; Stocker 2012). After attachment, some bacteria, possibly the “superior competitors” (Yawata et al. 2014), may become firmly associated with the aggregate (some even become embedded in the matrix). Others may detach again after a few hours of residing on the aggregates (Kiørboe et al. 2002), possibly adopting the “fugative strategy”, where less competitive species tend to travel more frequently to exploit new patches (Yawata et al. 2014). This leads to a temporal succession of baterial communities on particles and aggregates (Grossart and Simon 1998; Unanue et al. 1998; Knoll et al. 2001; Datta et al. 2016).

Additionally changes in AG BCC might be related to large aggregates scavenging smaller particles. As porous, sticky aggregates sink throught the water column, they may encounter smaller and slower sinking particles, which could be caught and encompassed in their matrix

(Li and Logan 1997, 2000). Thereby, the attached bacterial communities of the scavenged particle/ aggregate may also become part of the larger AG BCC (Engel et al. 2004).

Shifts in BCC on marine snow during sinking: Could it affect the efficiency of the BCP?

In the course of our sinking experiments (E-eddy and E-Sen), we observed an increase in Gammaproteobacteria and Flavobacteria and a simultaneous decrease of Alphaproteobacteria, which was not observed in the aggregates subjected to surface Flow water (E-upw). Since it is known that the capacity for POM degradation depends on the BCC (Enke et al. 2018), future experiments are needed to determine if this shift has an effect on the efficiency of the BCP. In fact, this shift has been shown previously to entail reduced bacterial respiration rates (Grossart and Ploug 2000). However, these comparisons are drawn on a very low taxonomic resoution (class level) and should thus be treated with caution. Nevertheless, if proven true, together with temperature and pressure, which have been suggested as the limiting factors for carbon degradation at depth (Grossart and Gust 2009; Iversen and Ploug 2013), shifts in the BCC during sinking of marine particles/ aggregates could be another factor increasing the efficiency of the BCP.

Differences in BCC depending on particle size

Marine, organic micro-particles and aggregates are hotspots of bacterial growth and activity (Azam et al. 1994; Ploug and Grossart 2000; Grossart 2010). We observed substantial variations in BCC on particles/ aggregates of different sizes (in our study PA vs. AG), which is in accordance with previous studies (Mestre et al. 2017a + b; Bizic-Ionescu et al. 2018). In this study, two reasons for this difference in BCC between PA and AG are likely:

1. The size of particles/ aggregates is crucial for determining BCC, because different sizes may represent differnt types of POM (Mestre et al. 2017a).
2. The experimental set up may have influenced differences between PA and AG. While larger aggregates, such as marine snow, could not be washed out of the FTRT, smaller

particles and aggregates (as found in the PA fraction) may have been replaced and thus originated from a different water mass.

As differences between PA and AG also prevailed at T_1, i.e. before the flow through was started, we can conclude that the sizes of the particles/ aggregates clearly affected bacterial colonization.

Single aggregate heterogeneity

Marine snow, i.e. macroscopic organic aggregates larger than 0.5 mm, are characterized by a gel-like matrix. Additionally, they can include a wide variety of (particulate) organic and inorganic matter, including e.g. phytoplankton cells, zooplankton (fecal pellets), transparent exopolymer particles (TEP), lithogenic material, etc. The composition of aggregates in turn affects bacterial colonization and therefore different types of aggregates will harbor different BCCs. In our experiments, BCC of single aggregates did not only vary among but also within all three experiments, indicating a high heterogeneity within the POM. While this complicates comparisons of individual AG BCCs between the different experiments, it reveals that aggregate colonization is largely impacted by the surrounding water in a random manner. Similar results have been found by Bizic-Ionescu et al. (2018) when comparing individual aggregates formed in the same FTRT, but in the freshwater Lake Stechlin. This indicates that BCC seem to be highly specific for individual aggregates, which may greatly differ in size and composition.

CONCLUSION

In this study we demonstrated that the shelf regions off Mauritania and Senegal were characterized by high aggregation volumes and aggregate sinking velocities (most likely due to ballasting), which can increase the efficiency of the oceanic BCP in this shelf region. The AG BCC was mainly determined at the beginning of the experiments. However, subjection to surface and deep water flow led to changes in AG BCC, suggesting exchange, e.g. possibly due to scavenging. The exposure of AG to vertical water masses, to simulate their settling through the water column, led to a decrease in Alphaproteobacteria, while Gammaproteobacteria and Flavobacteria increased. There were no comparable shifts in BCC when the AG BCC was subjected to horizontal water masses, to simulate their lateral export to the open ocean. Future studies should investigate if the observed shift in BCC during sinking can affect carbon turnover rates and thus influence BCP efficiency in the Canary Current system.

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SUPPLEMENTARY MATERIAL

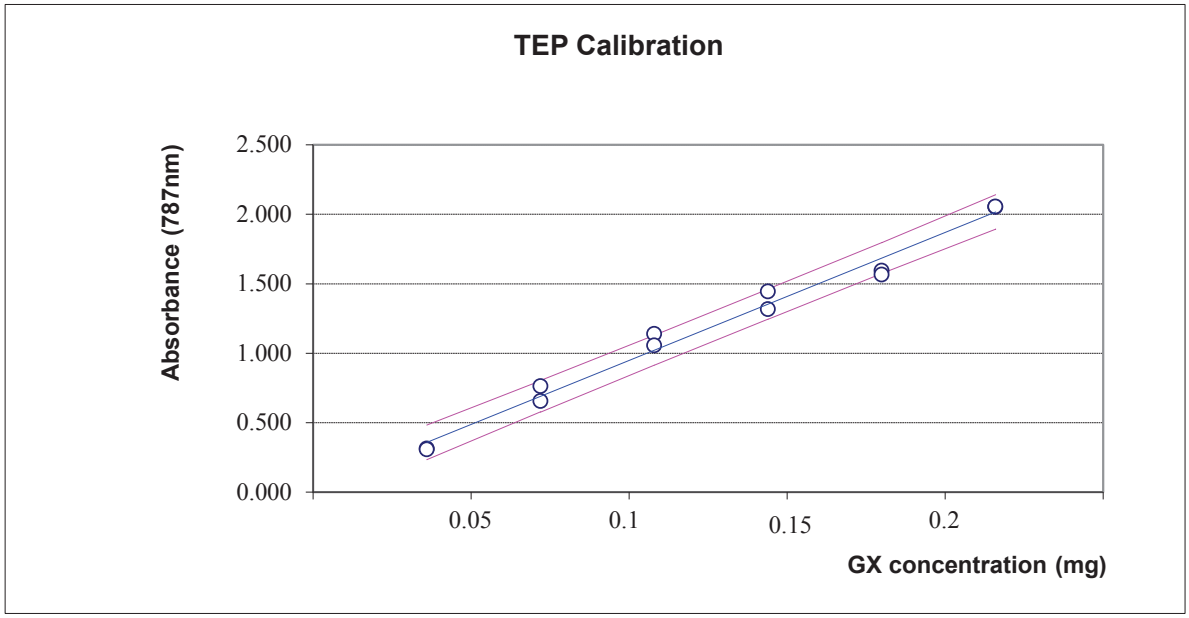

Figure S1. Example calibration curve obtained using the protocol of Engel (2009) with slight modifications (details see Materials and Methods section).

Table S1. All environmental parameters measured during the experiments. Stations refer to those in Bachmann et al. (2018).

EXP-Names	Station	Time	Type	NOx(µM)	NO_2(µM)	NH_4(µM)	NO_3(µM)	PO_4(µM)	SiO4(µM)	NP.ratio	DOC(µM)	N(µg L-1)	Ctot(µg L-1)	POC(µg L^{-1})	CN	TEP(µg L^{-1})
E-oligo	T0-1	T0	RT	0.00	0.06	0.12	0.00	0.09	1.14	0.00	333.00	NA	NA	NA	NA	NA
E-oligo	T1-1	T1/T2	FLOW	0.00	0.07	0.02	0.00	0.19	0.75	0.12	293.79	74.9	457.9	404.17	5.4	n.d.
E-eddy	T1-4	T0	Start	0.00	0.06	0.05	0.00	0.18	0.59	0.26	492.38	72.76	545.26	452.52	6.22	439.60
E-eddy	NA	T1	RT	0.00	0.00	0.24	0.00	0.13	0.35	1.84	607.73	68.08	462.12	456.54	6.71	NA
E-eddy	NA	T2	RT	0.00	0.00	0.15	0.00	0.19	0.35	0.77	864.11	73.65	581.27	486.64	6.61	NA
E-eddy	NA	T2	FTRT	25.28	0.08	0.61	25.21	1.18	7.43	22.04	367.35	57.65	405.85	324.57	5.63	NA
E-eddy	T1-6	T0	RES	26.61	0.13	0.00	26.49	1.61	7.67	16.55	472.09	4.76	54.76	40.25	8.46	n.d.
E-eddy	NA	T1	RES	25.72	0.13	2.00	25.60	1.64	7.28	16.90	332.82	NA	NA	58.468	NA	549.70
E-eddy	NA	T2	RES	25.31	0.34	0.31	24.97	1.12	7.70	22.87	339.58	63.53	348.04	314.2	4.95	NA
E-upw	T3-8	T0	Start	8.17	0.63	0.68	7.53	1.02	4.53	8.67	390.56	72.25	458.49	399.95	5.54	202.15
E-upw	NA	T1	RT	4.65	0.33	0.37	4.32	0.54	3.90	9.23	232.78	NA	NA	NA	NA	415.05
E-upw	NA	T2	RT	1.43	0.01	0.16	1.42	0.29	5.42	5.55	381.20	100.69	557.74	523.62	5.2	NA
E-upw	NA	T2	FTRT	0.35	0.00	0.03	0.35	0.23	2.58	1.70	329.21	63.88	469.59	535.74	8.39	NA
E-upw	T5-8	T1/T2	FLOW	0.13	0.03	0.33	0.10	0.31	0.04	1.52	312.87	27.02	209.01	196.82	7.29	47.33
E-upw	T6-4	T1/T2	FLOW	0.02	0.00	0.00	0.02	0.21	2.10	0.10	239.47	9.69	55.77	45.03	4.65	NA
E-upw	T6-6	T1/T2	FLOW	0.12	0.00	0.05	0.12	0.28	2.03	0.61	204.99	11.48	94.19	81.9	7.13	NA
E-upw	T6-7	T1/T2	FLOW	0.12	0.00	0.00	0.12	0.81	4.08	0.15	249.37	40.62	349.52	297.11	7.31	NA
E-Sen	T8-8	T0	Start	0.01	0.00	0.00	0.01	0.14	1.81	0.07	547.50	37.33	252.14	212.78	5.7	n.d.
E-Sen	NA	T1	RT	0.12	0.05	1.07	0.06	0.15	0.50	7.78	383.71	50.19	342.87	277.11	5.52	31.09
E-Sen	NA	T2	RT	0.04	0.03	0.36	0.01	0.09	0.26	4.33	197.22	41.34	313.21	268.25	6.49	134.95
E-Sen	NA	T2	FTRT	22.00	0.07	0.35	21.93	1.05	7.68	21.36	174.87	41.54	269.35	241.89	5.82	n.d.
E-Sen	NA	T1	RES	24.30	0.03	0.44	24.27	1.70	7.80	14.55	290.46	NA	NA	NA	NA	NA
E-Sen	NA	T2	RES	23.27	0.14	3.23	23.14	1.40	7.76	18.90	216.54	NA	NA	NA	NA	NA

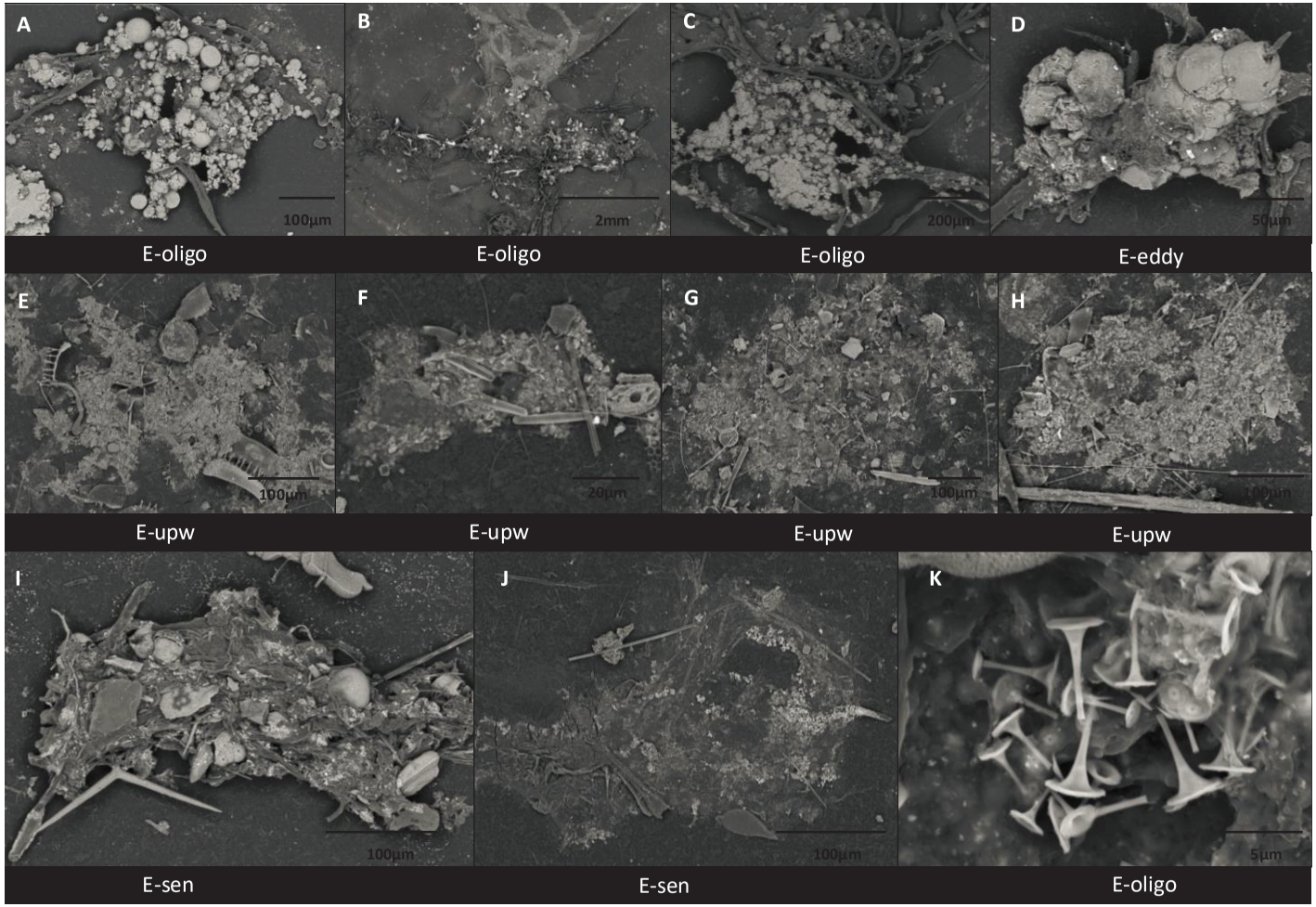

Figure S2. SEM overview pictures (A-J) of nine selected aggregates formed during the experiments as well as one detailed photo (K) of structures belonging to coccolithophores. E-oligo and E-eddy aggregates were characterized by coccoid objects, which may originate from foraminifera, whereas diatom and other debris is found within aggregates from E-upw and E-Sen.

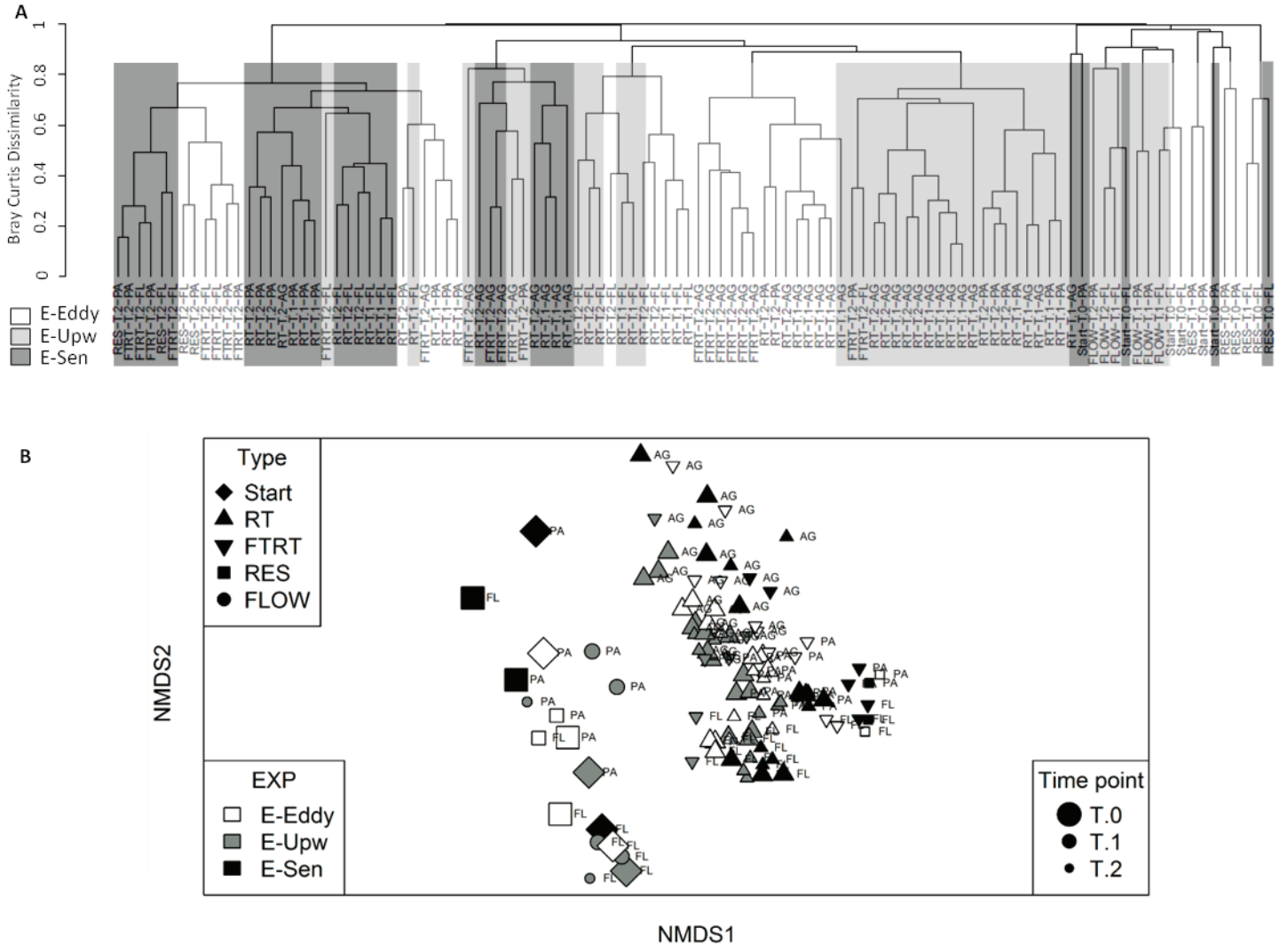

Figure S 3. Unweighted pair group method with arithmetic mean (UPGMA) and non-metric multidimensional scaling (NMDS) of FL, PA and AG bacterial communities.

CHAPTER IV

Particles in the spotlight: a measure of aquaculture- induced impacts on aquatic ecosystems

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ABSTRACT

Due to declining natural fish stocks, aquacultures are becoming increasingly important. However, coastal aquaculture practices are often environmentally unsustainable and lead to high particulate organic matter (POM) concentrations in the water column. However, so far the potential of particle characteristics to serve as indicators for aquaculture impact has not been investigated. In this context a reliable and cost efficient method to monitor *in situ* particle abundances was required. Furthermore, in Bolinao, Philippines, aquaculture practices have led to major fish kills due to recurring water column hypoxia and increased OM input to the sediments. However, so far the possible contribution of particle-attached microbial communities to water column hypoxia has not been investigated, yet. Therefore, during our field campaign in Bolinao in April/ May 2018, we tested the potential of POM characteristics to serve as indicator for aquaculture impact on coastal ecosystems. We characterized aquaculture derived particles and their relation to microbial activities and coastal biogeochemistry and established GoPros as functional particle detection systems. Aquaculture activities in Bolinao lead to higher masses of sinking particles, higher POC concentrations in the particles and more positive $\delta^{13}C$ values. On large particles, the aquaculture signature was visible in terms of lower bacterial Inverse Simpson indices and high relative abundances of fish gut derived bacteria. Although high sinking velocities of large particles (>0.5 mm) indicated a rather local impact of organic matter export, smaller particles may still be transported to adjacent coastal ecosystem. Microbial respiration rates of the particle-attached microbial communities suggested a strong O_2 drawdown by bacteria, which has the potential to contribute to the observed recurring hypoxic conditions in the waters off Bolinao. These results indicate that particle characteristics can serve as indicators the impact of aquaculture practices. Furthermore, bacteria attached to organic particles in aquaculture systems contribute to a substantial drawdown of O_2. Given the expected increase of aquaculture practices to ensure global food supply on the one hand and the importance of coastal ecosystems on the other hand, we urgently need a more sustainable approach to fish farming.

INTRODUCTION

Due to the dramatic decline of natural fish stocks, aquacultures are becoming increasingly important. Despite many advantages, such as the creation of jobs and provision of accessible food also in the developing countries (Edwards 2000), intensive aquaculture activities have been criticized for their numerous negative environmental impacts (Eng et al. 1989; Naylor et al. 2000). In coastal zones, e.g. habitat loss/ modification (Barbier and Sathirathai 2004), diseases (Reichardt et al. 2013) and use of antibiotics (Caruso 2016) are of concern. Additionally, waste from aquaculture activities can lead to increased dissolved (DOM) and particulate organic matter (POM) concentrations resulting in high microbial activities (Sarà et al. 2004; Reichardt et al. 2007).

DOM, POM and hydrogels make up the marine organic carbon (C) pool (Verdugo et al. 2004). Hydrogels are intermediates between DOM and POM and include for example the transparent exopolymer particles (TEP). While organic compounds smaller than 0.45 µm are defined as DOM, those exceeding 0.45 µm belong to the POM (Azam and Malfatti 2007). POM comprizes a variety of compounds, which include particles and aggregates. Particles are individual entities, such as single phytoplankton cells, while aggregates are characterized by several of these individual entities stuck together, often by a gel-like matrix (Riley 1963). Marine snow is an aggregate that exceeds the sizes of 0.5 mm. For simplification, in this manuscript, we use the term “particle” as a shortcut for particulate (organic) matter, which includes both particles and aggregates. In contrast the term “aggregate” solely comprizes aggregated material and will only be used in this manuscript if a particle was clearly identified as an aggregate (e.g. under the microscope).

While aquaculture derived DOM is dispersed, the particulate fraction tends to sink to the sea floor locally (Cromey and Black 2005), where the accumulating particles negatively impact the benthic biodiversity, e.g. due to benthic oxygen depletion (Holmer and Kristensen 1992, 1996; Brooks and Mahnken 2003). Since excess POM derived from aquaculture farms, and the subsequent increase in microbial activities in the surrounding water, can have such detrimental environmental effects, monitoring particle concentrations and fluxes *in situ* could provide useful information about environmental hazards of aquaculture activities.

For the study of deep sea particle fluxes in open ocean environments, the application of so called “particle cameras” (Ratmeyer and Wefer 1996) have proven helpful and provided

reliable information about particle abundances, sizes and even sinking velocities *in situ* (Honjo et al. 1984; Asper 1987; Nowald et al. 2006). More recently, also smaller cameras have been moored to conventional sampling devices, such as rosette samplers, in order to obtain reliable and cost efficient information on aquatic particle concentrations (Gorsky et al. 2000; Stemmann et al. 2012). If combined with sediment traps, the chemical properties of the particles can be assessed (Iversen et al. 2010). Hence, together these methods provide a great potential for monitoring major ecosystem variables, such as organic C fluxes and the cycling of OM. Knowledge about the fate of OM in an ecosystem further increases our understanding of food web structure and functioning as well as their responses to environmental changes. So far, the application of camera systems for *in situ* studies of aquaculture impacts on particulate matter concentrations is limited (Law et al. 2014). However, the application of action cameras (e.g. GoPros, GoPro, USA) provides a simple and cost efficient alternative to monitor *in situ* particle concentrations.

Organic particles are microbial hotspots characterized by high organic matter remineralization, which has major implications for biogeochemical element cycling (Bižić-Ionescu et al. 2014). Natural aggregates often result from algal blooms. As algal blooms decline, senescing cells increasingly excrete transparent exopolymer particles (TEP) (Engel 2000). TEP in turn promote aggregation and subsequent POM sedimentation, as they scavenge and stick together detritus, living cells, minerals and other materials upon their encounter in the water column (Passow 2002; Simon et al. 2002; Engel 2004).

In the microbial loop (Azam and Malfatti 2007) heterotrophic bacteria hydrolyze waste products and other POM into DOM and inorganic nutrients. By releasing the resulting bioavailable products into the surrounding water, new biomass production is supported, fueling the coastal food web from bottom up. The resulting high rates of microbial activities in the surrounding water and on the particles are of great concern. Microbes are able to draw down substantial amounts of oxygen often resulting in detrimental anoxic conditions. However, high dissolved oxygen concentrations are important for the survival of reared fish. Previous studies have shown that microbial communities attached to organic aggregates typically possess carbon specific respiration rates of around 0.1-0.2 d^{-1} (Ploug et al., 1999). Therefore, the microbial degradation of aquaculture-derived particles might not only result in benthic oxygen depletion (Holmer et al. 2002, 2003) but may also affect oxygen saturation in the overlying water column.

In Bolinao (the Philippines), intensive and extensive milkfish (*Chanos chanos*) mariculture has flourished since 1995 (San Diego-McGlone et al. 2008). Meanwhile, however, environmental health has been largely degenerated due to intense aquaculture activities (Reichardt et al. 2013). Fish kills, sometimes with considerable economic damage, occur repeatedly and often coincide with dying algal blooms and low oxygen concentrations (Azanza et al. 2005; San Diego-McGlone et al. 2008).

Previous research on aquaculture impact on POM dynamics in the area has mainly focused on microbial processes in the sediments in respond to the high OM input (Holmer et al. 2002, 2003). Results indicated that sediments below fish pens were enriched in OM by a factor of up to 4 and microbial oxygen consumption in the sediments was controlled by the OM input from the aquaculture activities (Holmer et al. 2002). Furthermore, it was concluded that anaerobic processes dominating OM degradation, was also stimulated by aquaculture activities (Holmer et al. 2003).

Despite the environmental impacts and major economic losses, resulting from aquaculture activities in Bolinao, mariculture impacts on water column POM dynamics has received little attention (Holmer et al. 2002, 2003; Reichardt et al. 2007). Especially microbial aggregate colonization and associated high respiration rates may contribute to recurring oxygen depletion in the water column and subsequent fish kills. Yet, they have not been quantified in the Bolinao area.

Therefore, during our field campaign in Bolinao in April/ May 2018, we will test the use of GoPros as functional particle detection systems. Secondly, we will characterize aquaculture derived particles and their relation to microbial activities and coastal biogeochemistry. Lastly we will quantify aquaculture effects on the coastal environment by using POM characteristics as an indicator.

MATERIALS AND METHODS

Sampling site description

Bolinao, which is located on the island of Luzon in the Philippines (Figure 1 B), is adjacent to the Guiguiwanen channel (Figure 1 A). The channel is characterized by high densities of fish cages, fish pens and mussle farms (Figure 1 C) and the most commonly farmed fish species is milkfish (*Chanos chanos*). The average depth of the channel is only 5 m, current speeds are up to 20 cms^{-1} and the muddy sea floor is covered by a thick layer of sulfidic sediments (Reichardt et al. 2007; White et al. 2007; San Diego-McGlone et al. 2008).

The complex water movements in the channel are governed by mainly semidiurnal tides (Rivera 1997). Simplifying, fresh sea water from the open ocean is supplied to the channel from the Western end during incoming tide (White et al. 2007; Geček and Legović 2010).

The first row of fish cages starts at the western side of the channel (Figure 1C). We chose our sampling station (16.38363639°N, 119.9156151°E) next to one of these cages in the front row due to the tidal movements in the bay. During outgoing tide, aquaculture influenced water from within the channel is transported past the selected sampling site at the western end of the channel, while during incoming tides fresh sea water is expected to flow past the sampling site. The water depth at the selected sampling station is 20 m and current speeds are around 5 cms^{-1} (White et al. 2007).

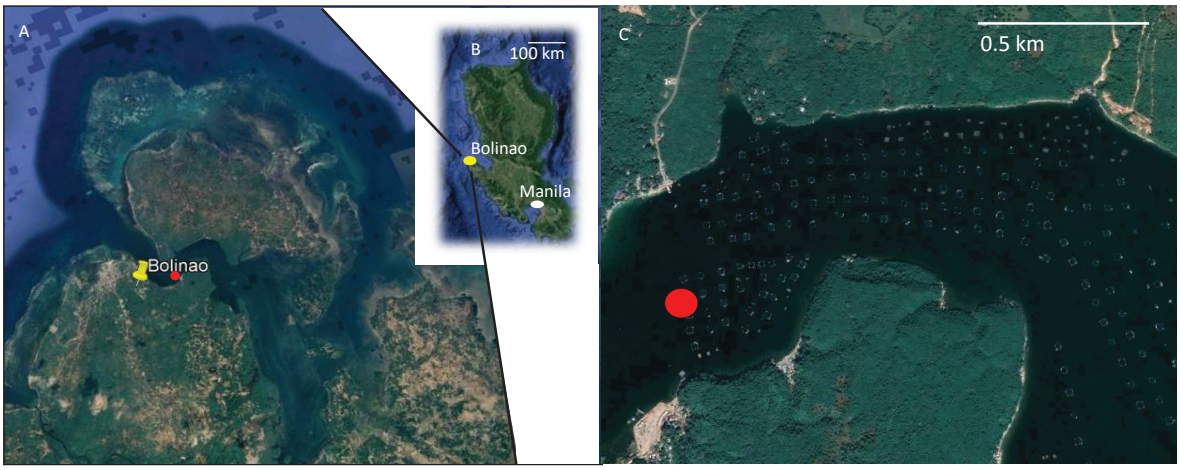

Figure 1: Bolinao, which is adjacent to the Guiguiwanen channel (A) is located North West of Manila (B). The sampling station (red circle) chosen for this study is at the North Western entrance of the channel. The channel is characterized by high aquaculture activities (C), whose structures are visible on satellite imagery (Images ©2018 CNES/Airbus © Googlemaps).

Since previous sampling campaigns in this region revealed a strong temporal variation of environmental parameters (Hassenrück et al., in prep.), sampling was carried out at only one location but in a high temporal resolution on 14 days over the course of three weeks in April/ May 2018 (Table 1). Whenever logistically possible, the incoming tide was sampled ca. two hours before high tide, whereas the outgoing tide was sampled ca. two hours before low tide (apart from 7th May where the outgoing tide was sampled just after high tide).

Table 1: Sampling was carried out for 14 days during April/ May 2018. The time of next high or low tides are displayed in bold. T9 is an outlier (#) as it was sampled just after high tide. Tides changed between neap and spring tides and water levels are shown in Figure S3.

Date	Sampling	Time	Tide	Abbrv.	Tide 1	Tide 2	Tide 3	Tide 4
27.04.2018	T1	08:07	incoming	T1-I	1:02*	**9:04**	11:54*	18:58
28.04.2018	T2	12:30	outgoing	T2-O	1:07*	8:43	**13:40***	20:06
30.04.2018	T3	14:25	outgoing	T3-O	0:43*	9:05	**16:10***	22:52
01.05.2018	T4	15:23	outgoing	T4-O	9:27	**17:12***		
02.05.2018	T5	07:58	incoming	T5-I	**9:52**	18:11*		
03.05.2018	T6	15:26	outgoing	T6-O	10:20	**19:08***		
04.05.2018	T7	08:50	incoming	T7-I	**10:51**	20:04*		
05.05.2018	T8	09:27	incoming	T8-I	**11:25**	20:59*		
07.05.2018	T9$^{\#}$	15:30	outgoing	T9#-O	12:49	22:32*		
08.05.2018	T10	11:50	incoming	T10-I	**13:42**	23:06*		
09.05.2018	T11	12:36	incoming	T11-I	**14:43**	23:31*		
10.05.2018	T12	14:00	incoming	T12-I	**15:51**	23:40*		
13.05.2018	T13	12:50	outgoing	T13-O	0:04*	7:55	**13:37***	19:47
14.05.2018	T14	13:50	outgoing	T14-O	0:02*	8:09	**14:52***	21:24

Environmental water parameters

At each sampling time point, environmental parameters, i.e. temperature, salinity, turbidity, pH, chlorophyll *a* (Chla) and dissolved oxygen (DO) concentrations were determined by using a multiprobe (Manta 2; Eureka, USA). Manta 2 measurements were carried out in intervals of 1 m from the surface to 20 m depth (corresponding to approximately 1 m above the sea floor to avoid influence of sediment nepheloid layer on turbidity measurements). Due to technical difficulties Manta recordings are only available from day T1-13

GoPro measurements of particle abundances and their size distributions

We used a GoPro Hero 4 (GoPro, USA) to determine the *in situ* abundances and sizes of marine particles. For calibration, the GoPro (in its water proof case) was attached to the bottom of a well-illuminated aquarium, which was filled with GF/F filtered sea water and covered with millimeter paper from the outside. Additionally, several millimeter paper covered cardboards were laminated and different points were marked on the cardboard by using pins. These cardboards were placed at known distances from the GoPro within the aquarium. In addition to calibrating the system, this set up allowed us to determine the minimum distance between object and GoPro lens so that objects appeared in focus on the pictures/ videos. Additionally, we could determine the fish eye distortion, which is especially prominent at the edges of the pictures and non-existent in the center. To test the ability of GoPros to record particles, three sinking marine aggregates (ESD 2-4 mm) made from roller tanks were placed into the water column at ca. 20 cm distance to the GoPro lens and video recordings were made. Image analysis was carried out using ImageJ (Version 1.52e, National Institutes of Health, USA). To calibrate the camera and convert pixels into the metric system, the number of pixels per mm was determined in triplicates.

For the field application, the GoPro was moored into a self-manufactured frame (dimensions see Figure 2) made of acrylic glass. The GoPro chamber was filled with GF/F filtered sea water to avoid particles appearing too close to the lens. A LED diving torch (Sea Dragon Mini 600, Sealife, USA) with up to 600 Lumen was attached on top of the GoPro chamber and used to illuminate the particle chamber.

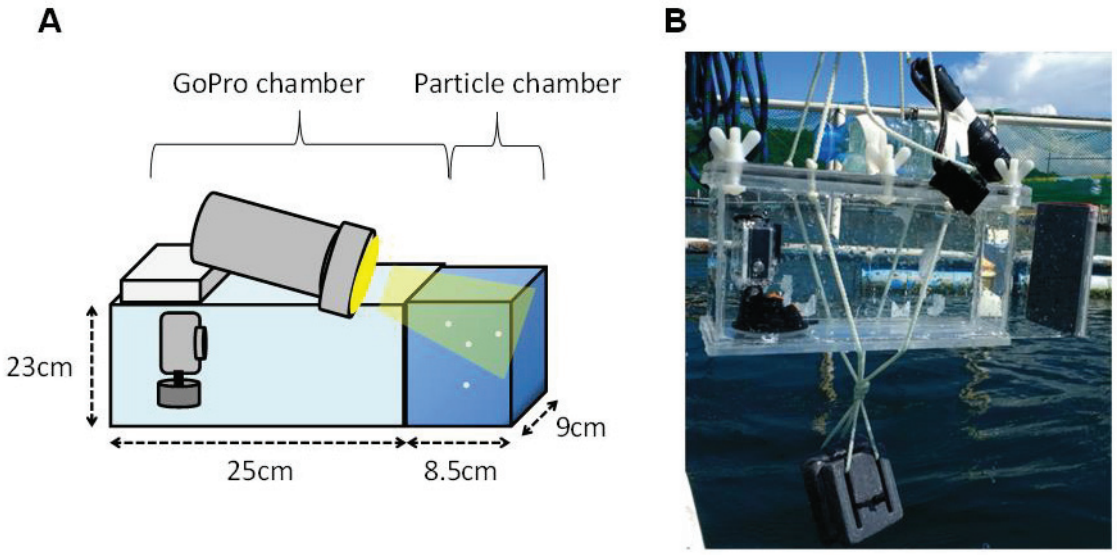

Figure 2: GoPro set (A) with a GoPro chamber filled with GF/F filtered sea water and a "particle chamber" where particles in the sample water could be recorded. Weights were attached to the bottom of the GoPro chamber (B) so that the device was floating upright in the water column with ropes attached to the four corners of the GoPro chamber to keep the system upright.

In this study, approximately 5 min underwater videos were recorded at 2, 5, 7 and 10 m depth. The GoPro recorded abundances and shapes of objects with a size >1 mm.

For particulate matter analysis, pictures were cropped so that only good quality selections without fish eye distortion were used for further processing. The selected pictures were converted into black and white images and a threshold was defined, by which objects (particles) were detected. The minimum and maximum detected areas were used to assign logarithmic size bins (see Jackson and Checkley 2011), where the upper value of the next bin was determined by multiplying the previous bin with 1.2. Particle number and volume spectra were calculated according to Jackson and Checkley (2011). For the volume spectra visualization, we used the diameter normalized volume spectra (nVd; see Jackson and Checkley, 2011). When plotting nVd as a function of logarithm of the equivalent spherical diameter (ESD) the area under the curve is proportional to the total particle volume.

Sediment trap application

Using two sediment trap arrays (model 28.200, KC Denmark, Denmark) with 4x ø 80 mm tubes each, the vertical mass flux was captured (Lalande et al. 2007). The bottom of each trap was equipped with a removable glass dish of the same size. Before deployment, the traps were filled with GF/F filtered sea water, whose salinity was increased by approximately 4 ‰.

Increased salinity within the traps allowed solely the collection of denser particulate matter. Traps were deployed at 10 m depth for 1 h. The collected material was used for bulk analyses of particulate C and nitrogen (N) content, stable isotope signature and bacterial community composition. To investigate aggregates, as one component of the particulate matter, in more detail, single aggregates were picked and their composition, sizes, sinking velocities, attached microbial communities and attached microbial respiration rates were determined.

CN content of particles

For bulk CN concentrations, the content of one sediment trap was filtered onto pre-weighed 47mm GF/F filters (Whatman, Dassel, Germany). Filters were rinsed with de-ionized water and dried at 40 °C (>48 h) before being re-weighed on a Mettler Toledo balance (sensitivity: 0.1 mg). Total carbon (C_{tot}) and total nitrogen (TN) concentrations were measured from one quarter of the GF/F filter using an Elemental Analyzer (EA-3000, EuroVector, Italy). The organic carbon fraction (POC) was measured after acidification of a second quarter of the GF/F filter with 1N HCl to remove all inorganic carbon. Particulate inorganic carbon (PIC) concentrations were obtained by subtracting POC from C_{tot}. CN ratios were calculated by dividing POC by TN.

Since POC (and TN) content can vary between different sizes of particles (Iversen et al. 2010), the collected material of one sediment cup was separated into large (L; diameter: ≥ 0.5 mm) and small (S; diameter: <500 µm) particles. L particles were picked out manually and counted. Their length and height was recorded and all L particles from the same trap and day were placed onto two pre-weighed 25 mm GF/F filters (Whatman, Dassel, Germany). Half of each filter was used for CN analysis and the second halves were prepared for POC analysis. The remaining content of the sediment trap (S fraction) was filtered onto a 47 mm GF/F filter. Further CN analysis was analogous to bulk CN analysis.

Stable isotope signature in particulate matter

Stable isotopes can be valuable markers for detecting the impact of aquaculture activity (Sarà et al. 2004). For the analysis of stable isotopes, the same filters as for CN bulk analysis were used. With 40 µg TN and 30 µg POC, the stable nitrogen isotope ratio ($\delta^{15}N$) and carbon isotopic ratio ($\delta^{13}C$) were determined using a mass spectrometer (Delta plus) coupled with Flash EA 1112 (Thermo Finnigan, USA). Results are expressed in the standard δ unit notation as $\delta X = [(R_{samples} / R_{reference}) - 1] \times 10^3$, where X is ^{13}C or ^{15}N, and R represents the ^{13}C: ^{12}C or

^{15}N:^{14}N ratios. The standard reference materials were Pee Dee Belemnite standard (PDB) for carbon and atmospheric N_2 for nitrogen.

Microscopic analysis of aggregates

Single marine snow aggregates were picked from one of the sediment cups, placed into Eppendorf tubes and stored at -20 °C until microscopic analysis. For aggregate pictures with a digital microscope (VHX 5000, Keyence, Japan), the aggregates were thawed and carefully transferred onto a petri dish.

Determination of bacterial communities on S and L particles and on single aggregates

Bacterial numbers will be determined via qPCR. Results from the previous year indicate a strong correlation between bulk POC concentrations and PA cell counts (Hassenrück, personal communication).

Bacterial community composition (BCC) on L particles was determined by manually picking out L particles (diameter ≥ 0.5 mm) from the collected material of one sediment cup analogous to the size separation for CN analysis. Their length and height was recorded and all L particles from the same trap and day were placed onto 5 µm Nucleopore TrackEtch polycarbonate membranes (Whatman, Dassel, Germany). The remaining content (S fraction) was filtered onto a second 5 µm polycarbonate membrane. Filters were stored and transported at -20 °C until DNA extraction.

Single aggregate (AG) BCC was determined from the same aggregates, for which respiration rates were measured. After determining size, sinking velocity and oxygen gradients via microsensor measurements, the aggregates were individually transferred into sterile Eppendorf tubes and stored and transported at -20 °C until DNA extraction.

DNA extractions of S and L fractions as well as AG samples were carried out according to Bossier et al. (2004); Nercessian et al. (2005) and Wang and Wang (2012) with several modifications (see full protocol in supplementary material). The S and L filter samples were further purified using NEB Monarch® PCR & DNA Cleanup Kit (New England Biolabs, USA) according to the manufacturer's instructions. Samples were stored at -20 °C until sequencing.

Using the Nextera XTv2 kit (Illumina, USA), amplicon sequencing libraries of S, L and AG samples were prepared at the Max Planck Institute for Marine Microbiology with the universal prokaryotic primer pair 515F-Y (5'-GTGYCAGCMGCCGCGGTAA-3') and 926R

(5'-CCGYCAATTYMTTTRAGTTT-3') targeting the V4-V5 hypervariable region of the 16S rRNA gene (Parada et al. 2016). Sequencing was performed on an Illumina MiSeq using V3 chemistry (Illumina) in a 2x 300 base pair paired-end run at CeBiTec, Bielefeld.

Demultiplexed sequences provided by CeBiTec were primer-clipped using cutadapt version 1.17 (Martin 2011). Further sequence processing steps were conducted in R version 3.5.1 (R-Core-Team 2018) using the package dada2 version 1.9.3 (Callahan et al. 2016) with default parameters unless indicated otherwise. Sequence quality filtering was conducted at a maximum expected error rate of 4 and a minimum length of 235 and 195 bp for forward and reverse reads, respectively. After error learning, denoising, merging and chimera detection, operational taxonomic units (OTUs) were defined as unique sequence variants within the range of 362 and 399 bp that occurred at least twice in the data set. These sequences were taxonomically classified using the online tool SILVAngs (https://www.arb-silva.de/ngs/; date accessed: 07.09.2018) and the SILVA ribosomal RNA gene database version 132 as reference (Quast et al. 2013). Chloroplasts, mitochondria and operational taxonomic units (OTUs) unclassified on phylum level and OTUs with a sequence similarity of less than 93% to the reference data base were excluded.

Based on the 16S gene of the chloroplast, the presence/ absence of eukaryotic phytoplankton genera was inferred as described in (Bachmann et al. 2018). Therefore, chloroplast 16S sequences were aligned against a customized reference database consisting of chloroplast sequences from the NCBI refseq database (date accessed: 03.06.2018). Sequences with similarity >/ = 93% to known phytoplankton taxa were analyzed further and a detection threshold of at least 10 sequences was chosen to achieve a higher confidence in the presence of the respective phytoplankton taxon.

Flow chamber: Equivalent spherical diameters and sinking velocities

Single aggregates were transferred from a sediment cup into a vertical flow system filled with sterile filtered sea water (Ploug and Jørgensen 1999). Their lengths, heights and widths were recorded using a horizontal microscope (Müller Optonics, Germany). Their volume was calculated assuming an ellipsoid shape and their diameter was calculated as equivalent spherical diameter (ESD) of a sphere with the same volume as the ellipsoid (Iversen et al. 2010). To determine their sinking velocities, the upward flow in the vertical flow system was adjusted with a needle valve (Ploug and Jørgensen 1999). Triplicate measurements of the upward flow rates were made for the aggregates, once they were floating one diameter above

the net, where their sinking velocity was balanced by the upward-directed flow velocity. The sinking velocity was calculated by dividing the flow rate by the cross-sectional area of the flow system.

Microelectrode respiration measurements

Oxygen measurements were performed according to Ploug et al. (1999, 2008). Therefore, oxygen concentrations were measured with a 20 µm step size from above the aggregate, through the diffusive boundary layer, to the center of the aggregate. Using a microelectrode (Unisense, Denmark) with a tip diameter of 10 µm triplicate oxygen profiles were measured (Revsbech 1989). A diffusion-reaction model to calculate the oxygen uptake rates and to estimate the apparent diffusivity inside aggregates was applied to measure the O_2 consumption rates in each aggregate (Ploug et al., 1997, 2002). Carbon respiration rates were calculated from oxygen consumption rates by assuming a respiratory quotient of 1 mol O_2 to 1 mol CO_2 (Ploug and Grossart 2000). Since aggregate POC concentrations could not be determined from the same aggregate (as it was used for sequencing), the average POC concentrations of the L fraction (see CN content of S and L fraction) of the respective day was used to calculate carbon specific respiration rates.

Statistical analyses

All statistical analyses were run in R studio using the core distribution (R-Core-Team 2015) and additional packages, such as *vegan* (Oksanen et al. 2015) and *gplots* (Warnes et al. 2016).

Environmental parameters and total particulate matter abundances (determined by GoPro recordings) of T2-T13 were displayed in a principal component analysis (PCA). Since pH values were missing for T13, pH was added using the function *envfit*.

Differences in CN content of aggregates collected during the incoming vs. the outgoing tide and of different sizes were tested using a t-test or Wilcox test, the non-parametric equivalent, if applicable. Differences in carbon specific respiration rates between incoming and outgoing tide were assessed using a Wilcox test.

Alpha diversity was determined as numbers of OTUs (nOTUs) and using the Inverse Simpson Index. The Inverse Simpson Index was calculated without prior rarefying to equal library sizes because rarefaction curves were saturated at the obtained sequencing depths (Chao et al. 2014).

Pairwise BC dissimilarities were used for cluster analysis (unweighted pair-group method using arithmetic average, UPGMA). Ordination of S, L and AG sequences was carried out using non-metric multidimensional scaling (NMDS) on relative sequence abundances of OTUs.

The relationship between single aggregate microbial community composition and tidal phase, carbon specific respiration rate, aggregate volume and sinking velocity were tested via redundancy analysis (RDA). Prior to RDA, rare OTUs were removed by filtering the data set to OTUs occurring with a proportion of at least 0.1% in at least 2 samples. This OTU removal did not affect beta diversity trends (mantel test, $r = 0.97$, $p = 0.001$). Furthermore, sequence counts were centered log-ratio (clr) transformed using the median of 128 monte-carlo instances produced by the aldex.clr function. After RDA, the correlation of individual OTUs and the predictors, which explained the highest variance in the RDA, were calculated using aldex.corr.

Future potential of this data set

The data set of this manuscript still has a lot of potential, and continues being analyzed as this manuscript is being published. Future analyses will include for example qPCR of the 16S gene for estimates of bacterial abundances and of marker genes for pathogenic *Vibrios*. Additionally, carbon fluxes (which will be estimated from particle abundances, single aggregate sinking velocities and POC content) are expected to provide further proof for the export of high amounts of organic carbon to the sediments. Thirdly, during the current analysis of this data set environmental parameters and tidal amplitude suggested that the results of the sampling campaign can be sub-structured in three (or at least two) distinct temporal phases. This may allow for future (statistical) analyses on temporal differences in particle abundances, characteristics and their BCCs.

RESULTS

Environmental parameters

Environmental parameters varied substantially over the sampling period. Especially, surface temperatures, salinities, dissolved oxygen (DO) and chlorophyll a (Chla) concentrations decreased towards the end of the sampling campaign (Supplementary Figure S1).

Environmental parameters at 2, 5, 7 and 10 m depth were ordinated in a PCA (Figure 3) and raw values are available in Supplementary Table S 1. In the PCA the first two principal components (PC1 and PC2) explained 80% of the variability in the data set. PC1 was influenced by turbidity (Turb), temperature (Temp), Chla and salinity (Sal) while PC2 and PC3 strongly positively correlated with DO and total particle abundances (GoPro), respectively. Temperature and Chla concentrations decreased with depth. Over the sampling period salinity and pH tended to decrease and increase, respectively. Although not as clear because of variable influences by sampling time, PC1 seems to be an indicator for tidal effects, generally ordinating outgoing samples in the negative and incoming in the positive range. The outgoing sampling T9#-O and T13-O were very different from the remaining outgoing samples due to relatively high oxygen concentrations and in contrast to T3-O, T4-O and T6-O they additionally had lower Chla concentrations and were characterized by lower salinities and turbidities. On T5-I and T7-I, 2 and 5 m samples clustered with the outgoing samples because of higher salinities, lower DO and higher Chla concentrations.

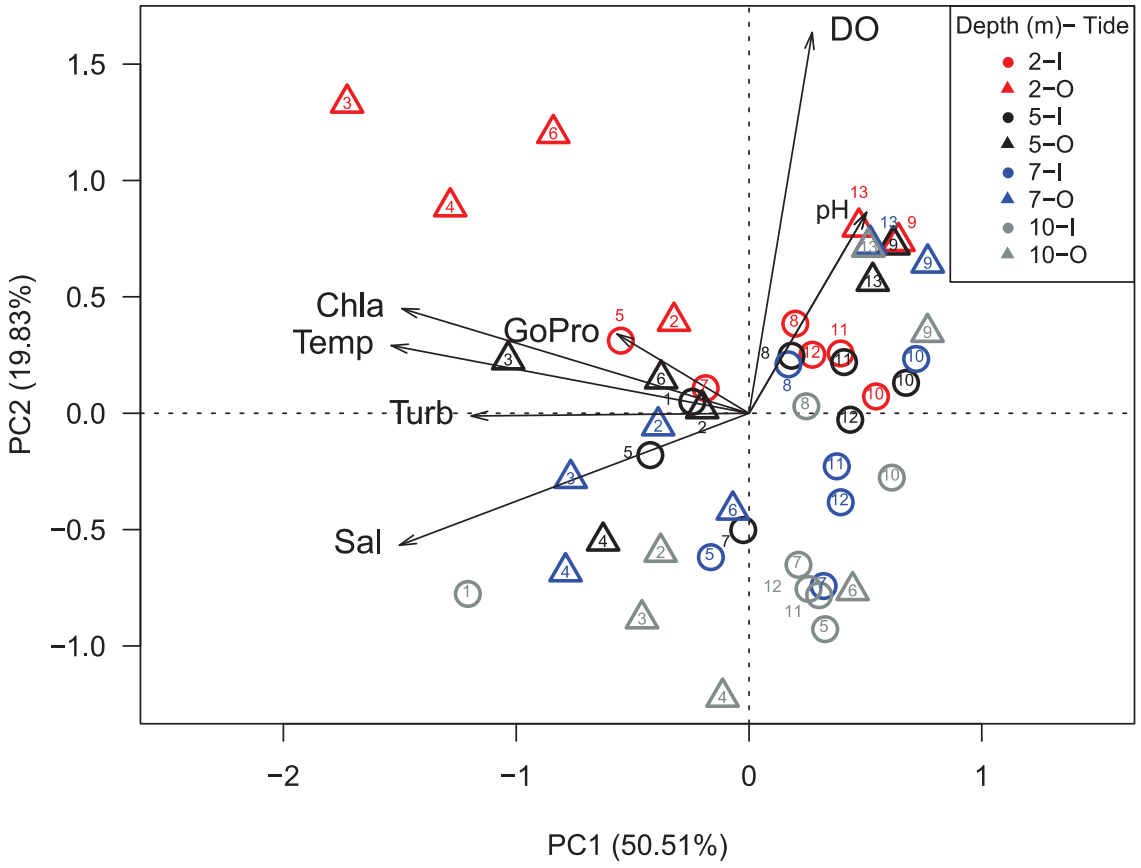

Figure 3: Principal component analysis (PCA) with environmental parameters measured at 2, 5, 7 and 10 m depth. They include turbidity (Turb), salinity (Sal), temperature (Temp) chlorophyll a (Chla), dissolved oxygen (DO) and total particle abundances measured with the GoPro system (GoPro). Numbers in/ next to symbols indicate sampling days (s. Table 1). Manta data at 2 and 7 m depth for sampling day 1 is not shown as GoPros only recorded at 5 and 10 m on the first day. Manta recordings are only available for sampling T1-13, therefore GoPro data for T14 is also omitted.

GoPro as functional particulate matter detection system

Testing the GoPro system, the minimum distance between an object (e.g. particle) and the GoPro lens was 20 cm. The size range of GoPro recorded particles was equal to that of L particles found in the sediment traps. The particle abundance trends are similar to those of the Secchi depth data (Supplementary Figure S 3). This suggests that GoPros can be reliably used to detect particle abundances and sizes in the water column.

Particle abundances determined by GoPro measurements

A total of 6:15 h of GoPro videos were analyzed for particle abundances and their sizes. Total particle abundances ranged between 4-187 particles L^{-1} and they tended to be higher during outgoing than incoming tide (Figure S 2). In general, particle abundances and Secchi depth (Figure S 3) showed similar trends over the sampling period.

Highest total particle abundances at 2, 5 and 7 m were observed on days T2-O, T3-O, T11-I and T12-I. At T5-I, T6-O, T7-I and T10-I abundances were very low with the same patterns over depths.

Lowest outgoing particle abundances at 5-10 m depth with only 8 particles L^{-1} were observed on T4-O. Afterwards, there was a steady increase among the outgoing particle abundances until they reached around 50 particles L^{-1} on T14-O.

Particle number spectra (Supplementary Figure S 4) indicate that the size range of recorded particles was between 0.98 and 8.6 mm. The lower boundary was defined by optical limitation and the upper boundary by the low occurrences of larger particles. Particle number spectra lay between 0 and 3.79 particles cm^{-4}. There were higher numbers of small particles and depth profiles generally looked similar between different sampling dates, indicating the reproducibility of GoPro data. There were some changes in particle number spectra over depth, which may have been caused by environmental variability (e.g. T4-6). The particle number spectra indicate that highest frequency of smallest particle bins was responsible for high total particle abundances on the first four sampling days.

Particle volume spectra (Supplementary Figure S 5) varied between 0 and 0.000045 ppm. In general, particles with an ESD of around 0.127 cm contributed to the highest particle volume. Over the sampling period highest particle volumes were observed on the first four days. Very similar (and lowest) particle volume spectra were observed between T4-O and T7-I and on T9#-O and T10-I. Afterwards the particle volume spectra increased again slightly.

CN content of particles

The median weight of material collected in the trap (mass flux) was higher in the outgoing bulk samples than in the incoming (Figure 4). In the bulk particulate matter, C_{tot} varied between 1.6 and 11.6 µg mg^{-1} dry weight. Ranges of POC were very similar (between 2.3 and 11.5 µg mg^{-1}). The highest C_{tot} and POC concentrations per mg sample occurred on T11-I. Apart from on T11-I, C_{tot} and POC per mg sample tended to be higher in the outgoing samples. However, there were greater variations in POC during both incoming and outgoing tides than in C_{tot}. In the bulk samples, PIC ranged between 0 and 2.4 µg mg^{-1} with the highest PIC concentration occurring during outgoing tide. Medians of C_{tot} and POC were higher during outgoing (5.7 µg mg^{-1} and 5.4 µg mg^{-1}, respectively) than during incoming tide (6.4 µg mg^{-1} and 6.2 µg mg^{-1}, respectively). Differences between incoming vs. outgoing tides were neither significant for C_{tot} (Wilcox, p=0.1), POC (t-test, p=0.65) nor for PIC (t-test, p=0.2).

Although, differences between incoming and outgoing tides were insignificant for TN concentrations (Wilcox, p=0.45), they showed a higher variability during the outgoing tide. In the bulk material, TN ranged between 0.3 and 1.4 µg mg^{-1}.

Results from the size-fractionated samples matched the bulk samples and indicated that L particles on average contained higher C_{tot}, POC and PIC concentrations per mg dry weight than S samples. The high C_{tot} and POC concentrations at T11-I were found in the L fraction.

C_{tot} (t-test, p= 0.0005), POC (t-test, p= 0.0005), and PIC (Wilcox, p= 0.0037) concentrations significantly differed between the two particle size classes (S and L) with higher carbon content in the L size classes. In contrast, there were no significant differences in the concentration of TN (Wilcox, p= 0.39) between S and L particles because TN concentrations varied greatly among S particles.

Median CN ratios of incoming (around 8) samples were slightly, but insignificantly (t-test, p= 0.9), higher than of outgoing (around 7) samples (Table S1). Highest CN ratios were observed towards the end of the sampling campaign, on T7-I, T11-I, T13-O and T14-O.

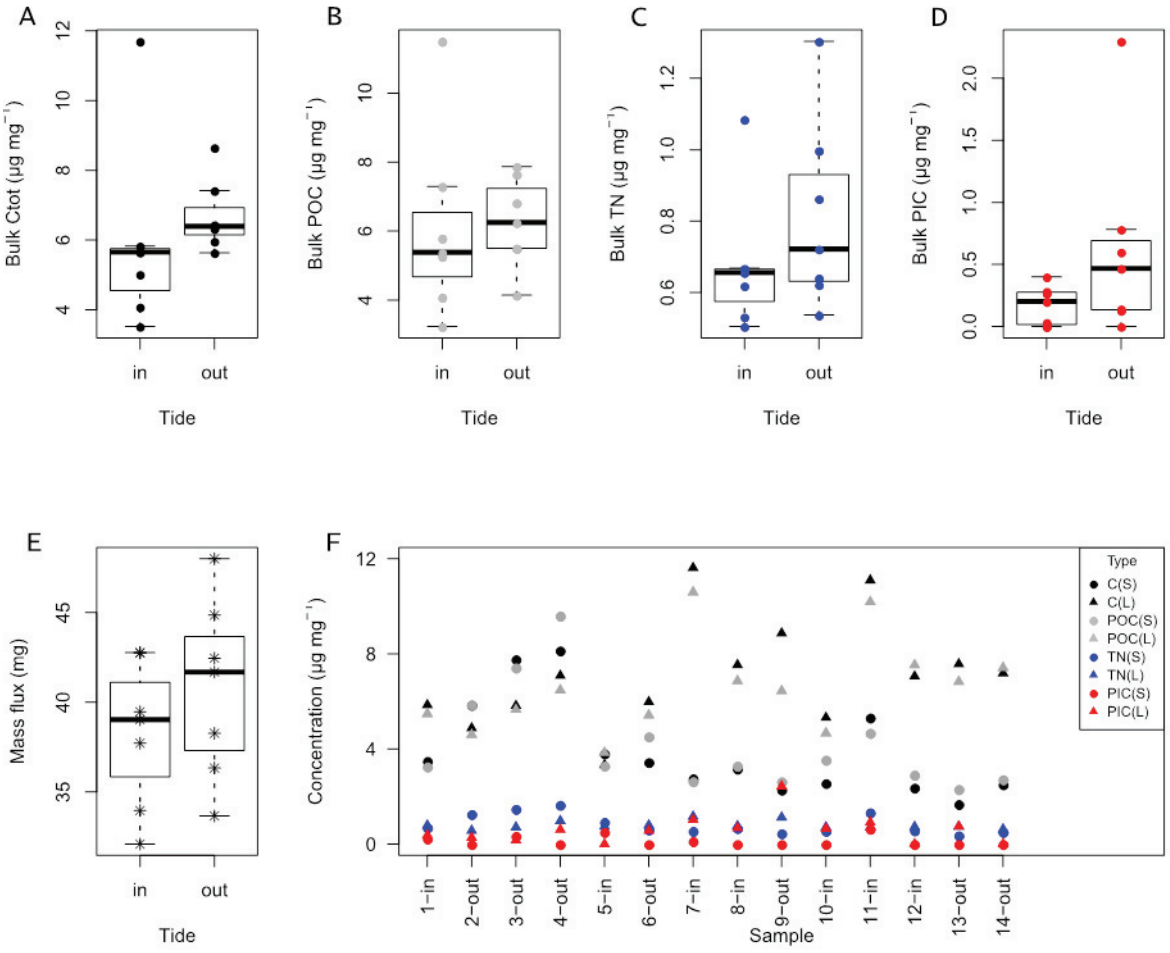

Figure 4: C_{tot}, POC, TN and PIC content of particles at incoming and outgoing tides (A-D) and mass fluxes (E). Variations in C_{tot}, POC, TN and PIC content in the S and L size fractions are shown over the sampling period (F).

Stable isotope signature of particles

The C and N isotopic values are displayed in Figure 5. The average C isotopic value of incoming (-23.1 ‰) were lower than those of outgoing (-22.4 ‰) samples but higher than the average of fish pellets (-26.2 ‰). Average N isotopic values were very depleted and similar for incoming (2.1 ‰), outgoing (2.2 ‰) samples and fish pellets (2.1 ‰). Although there were no significant differences between incoming and outgoing $\delta^{13}C$ (t-test, p=0.16) nor $\delta^{15}N$ (t-test, p=0.7) values, differences between incoming and outgoing samples tended to be reflected in $\delta^{13}C$ values. Lowest $\delta^{15}N$ values (T4-O and T5-I) were at the times of lowest DO concentrations observed during the sampling period.

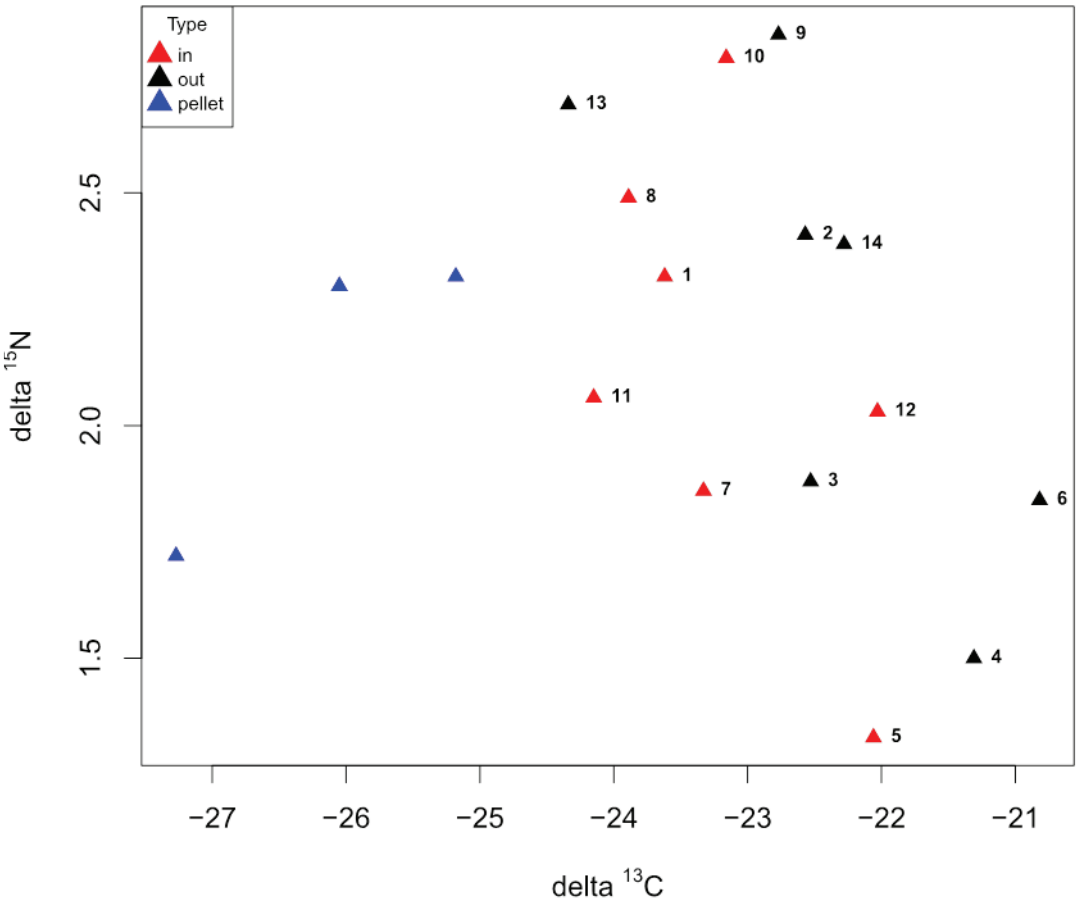

Figure 5: $\delta^{13}C$ and δ15N values of bulk particulate matter and fish pellets.

Bacterial alpha diversity

Four samples (S: T3-O, T4-O, T6-O; L: T9#-O) contained <5000 sequences and were excluded from further analysis in this PhD thesis. These samples will be resequenced and analyzed. The remaining samples contained between ca. 9000 and 600,000 sequences. Sequencing depth tended to be higher for single aggregates than for the S and L fraction.

Median nOTUs was highest in L samples, while median Inverse Simpson indices were similar in all fractions (Figure S 6). The median Inverse Simpson index was 62 and ranged between 3 and 240. Both minimum and maximum Inverse Simpson Indices were found in the AG fraction.

In the S fraction, both nOTUs and Inverse Simpson index were higher during the outgoing tide while in L and AG samples, Inverse Simpson indices were lower during outgoing tides.

The inverse Simpson index of the L fraction was similar from T1-5, after which a sudden decrease in alpha diversity was observed. Afterwards the Inverse Simpson index steadily increased again until highest alpha diversities were observed on T10. On sampling T11the Inverse Simpson index had crashed again and maintained similar levels until the end of the sampling period.

Bacterial beta diversity

Single aggregate bacterial communities were very heterogeneous (Figure 6). S and L attached communities formed a more homogeneous sub-cluster within the AG samples. There was a trend that AG communities clustered according to sampling day. Single aggregate bacterial communities were distinct from the S and L communities. Within the S and L fraction, BCC tended to cluster according to tide.

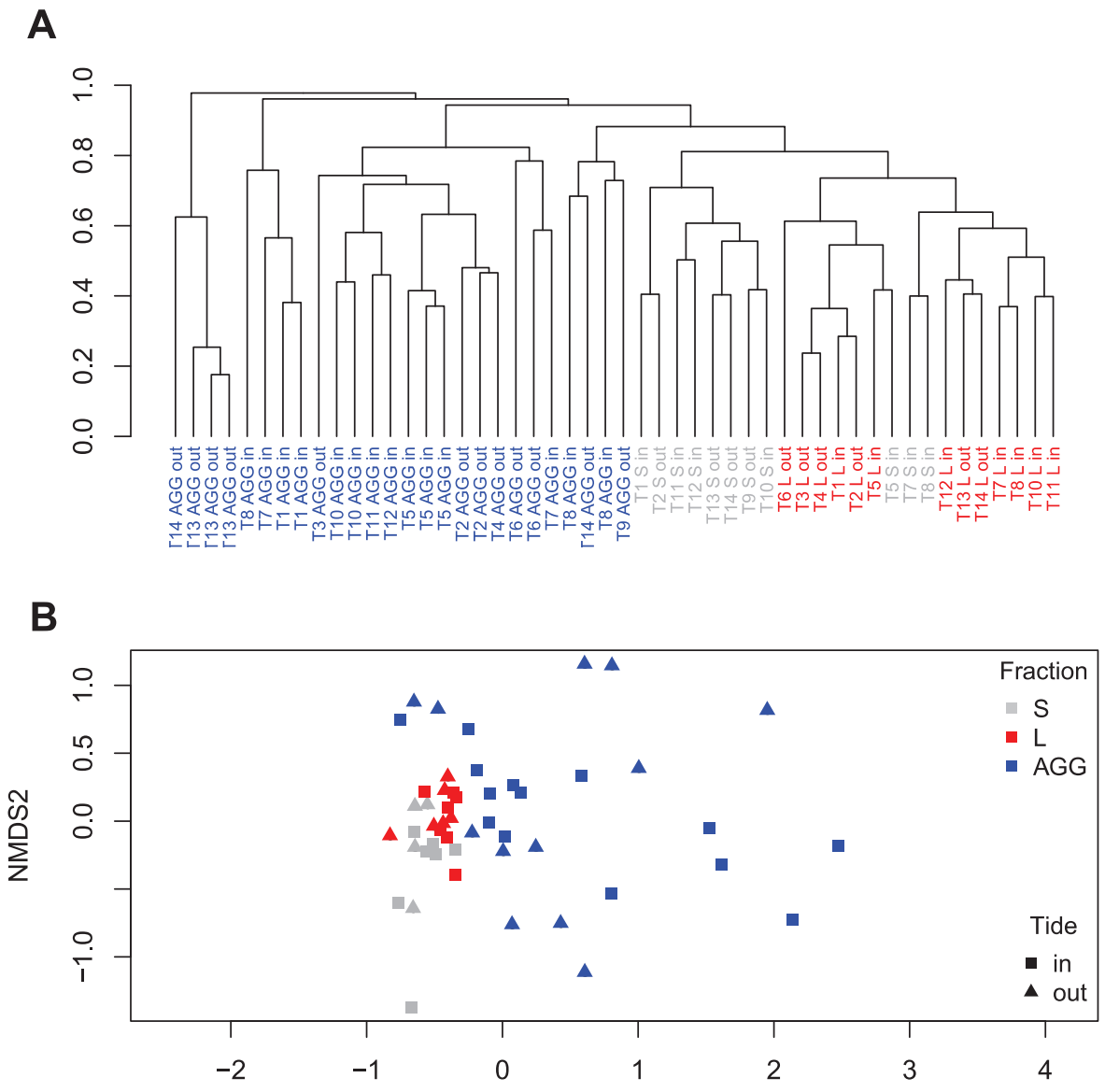

Figure 6: UPGMA and NMDS of BCC of small (S) and large (L) fraction and single aggregates.

Bacterial community composition of S and L particles

The most common phyla among S, L and AG samples in this study were Proteobacteria (63%), Bacteroidetes (16%), Planctomycetes (7%), Firmicutes (3%), Fusobacteria (3%) and Cyanobacteria (2%). Proteobacteria were dominated by Gammaproteobacteria and Alphaproteobacteria.

Differences between BCC (at order resolution) of S and L particles were mainly due to variations in the dominance of certain groups of bacteria (Figure 7). In S particles there was a tendency towards higher relative abundances of Cyanobacteria and Arenicellales (Gammaproteibacteria). L particles were characterized by higher relative abundances of

Erysipelotrichales (Firmicutes), Vibrionales (Proteobacteria) and Fusobacteriales (Fusobacteria).

Erysipelotrichales were present in especially high relative abundances in the L fraction from T1-T8 and at T11-I during both incoming and outgoing tides. Their relative abundances peaked on T6-O and this dominance was reflected in the lowest Inverse Simpson index of the L fraction during the sampling period. Their relative abundances decreased again (and alpha diversity increased) until T10-I. Towards the end of the sampling period Erysipelotrichales were only present at very low relative abundances. Highest relative abundances of Fusobacteriales occurred in the L fraction at T11-I. Similar to the Erysipelotrichales, their relative abundances decreased towards the end of the sampling period (i.e. T12-T14). In contrast the relative abundances of Vibrionales in the L fraction peaked on T14-O.

Highest relative abundances of particle degrading Planctomycetales (Planktomycetes) occurred in the S fractions on T5-I and T8-I. Together with the co-occurring high relative abundances of Flavobacteriales (Bacteroidetes) and Rhodobacterales (Proteobacteria), the dominance of Planktomycetes at T8-I were reflected in the lowest Inverse Simpson index of the S fraction. Arenicellales tended to increase in relative abundances within the S fraction towards the end of the sampling period.

RDA analysis revealed that only a very small proportion of variation in the S and L fractions could be explained by POC and TN content or tide.

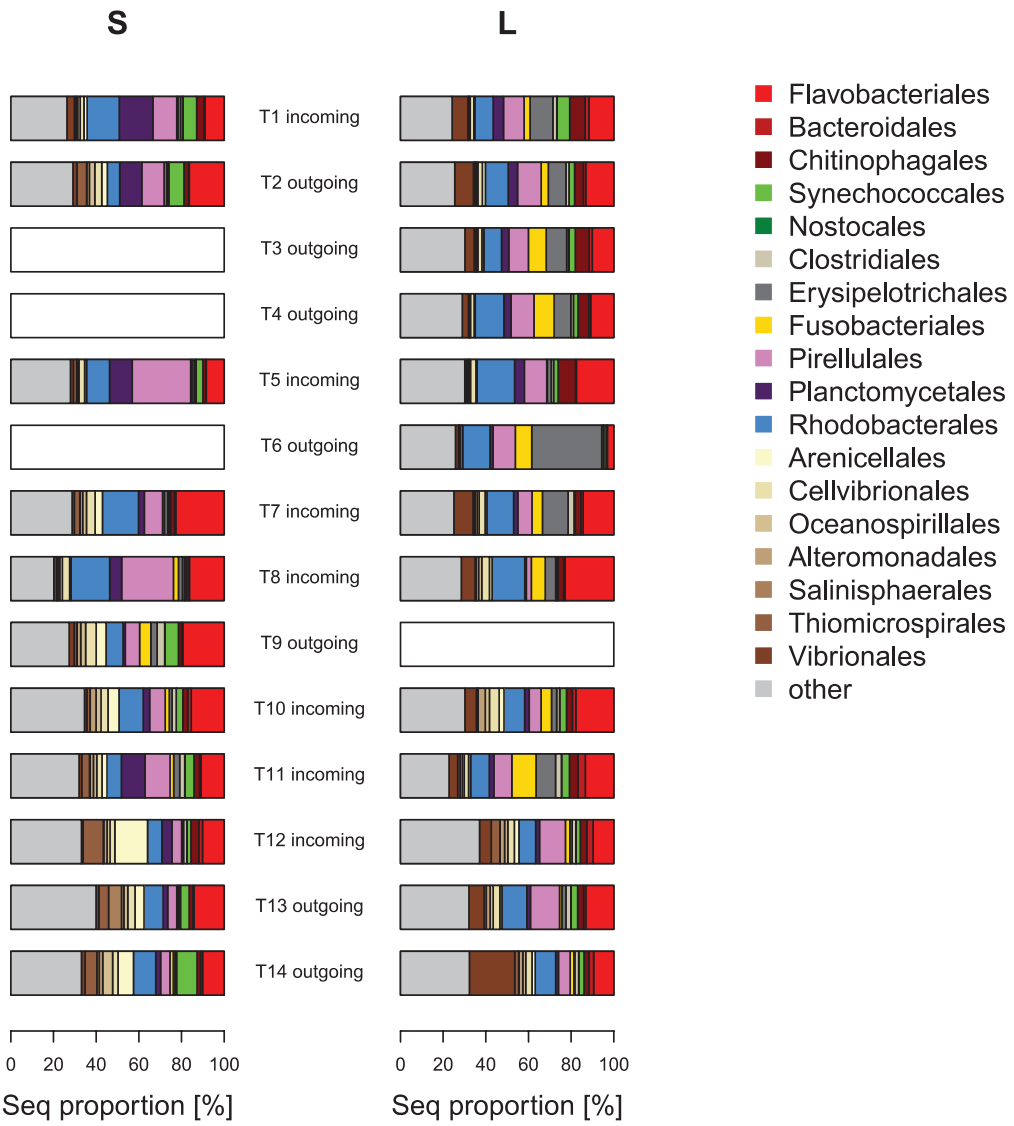

Figure 7: Bacterial community composition of small (S) and large (L) fraction (bulk samples). Empty bars indicate samples with <5000 sequences in the first sequencing run. Hence they will be resequenced.

The four major types of aggregates

The bulk particles were inspected in more detail and therefore single aggregates were picked for microscopic analysis. This revealed four major types of aggregates (Figure 8): green microalgae (A), plastic (C) and macroalgae (D) dominated aggregates as well as undetermined brown aggregates, which might originate from fish feces (B). Some aggregates contained food particles (not shown) and sediment grains (e.g. black dots in Figure 8A). Both food particles and sediment grains (inorganic material) increased the sinking velocities of aggregates. Some aggregates were larger (e.g. A), reaching sizes of up to 2.5 millimeters. The majority of particles, however, was between 1-1.5 mm in diameter.

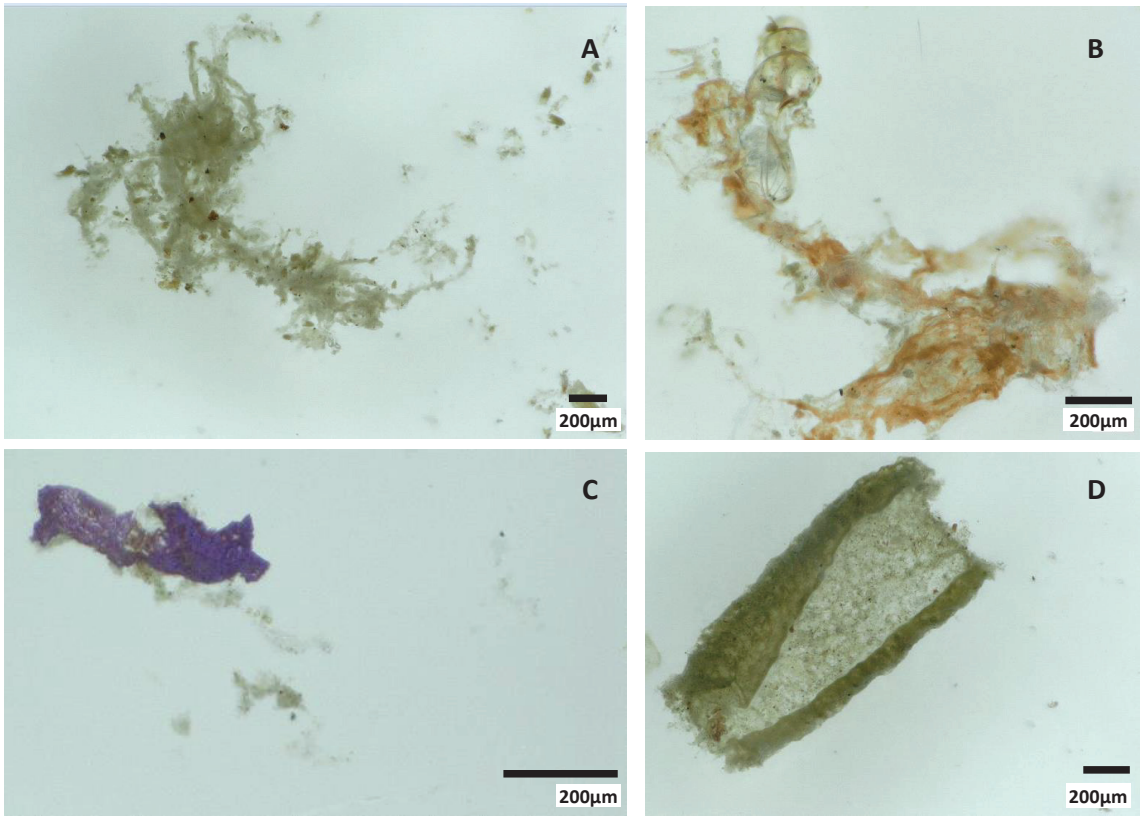

Figure 8: Photos of typical single aggregates (A, B), which appeared greenish (A) or brownish (B), probably resulting from the incorporation of algae and fish feces, respectively. Plastic (C) and larger algal particles (D) were also observed to form the basis of aggregates.

Flow chamber analysis and microelectrode respiration measurements

Mean sinking velocities of aggregates were 132 m d^{-1} and ranged between 80 and 286 m d^{-1} (Figure 9). Microbial respiration rates ranged between 2 and 32 µmol agg^{-1} d^{-1} (Supplementary Table 2). C specific respiration rates were between 0.09 and 1.75 d^{-1} and median C specific respiration rates were slightly higher during outgoing tide (due to the three outliers). However, differences were insignificant (Wilcox, p= 0.25).

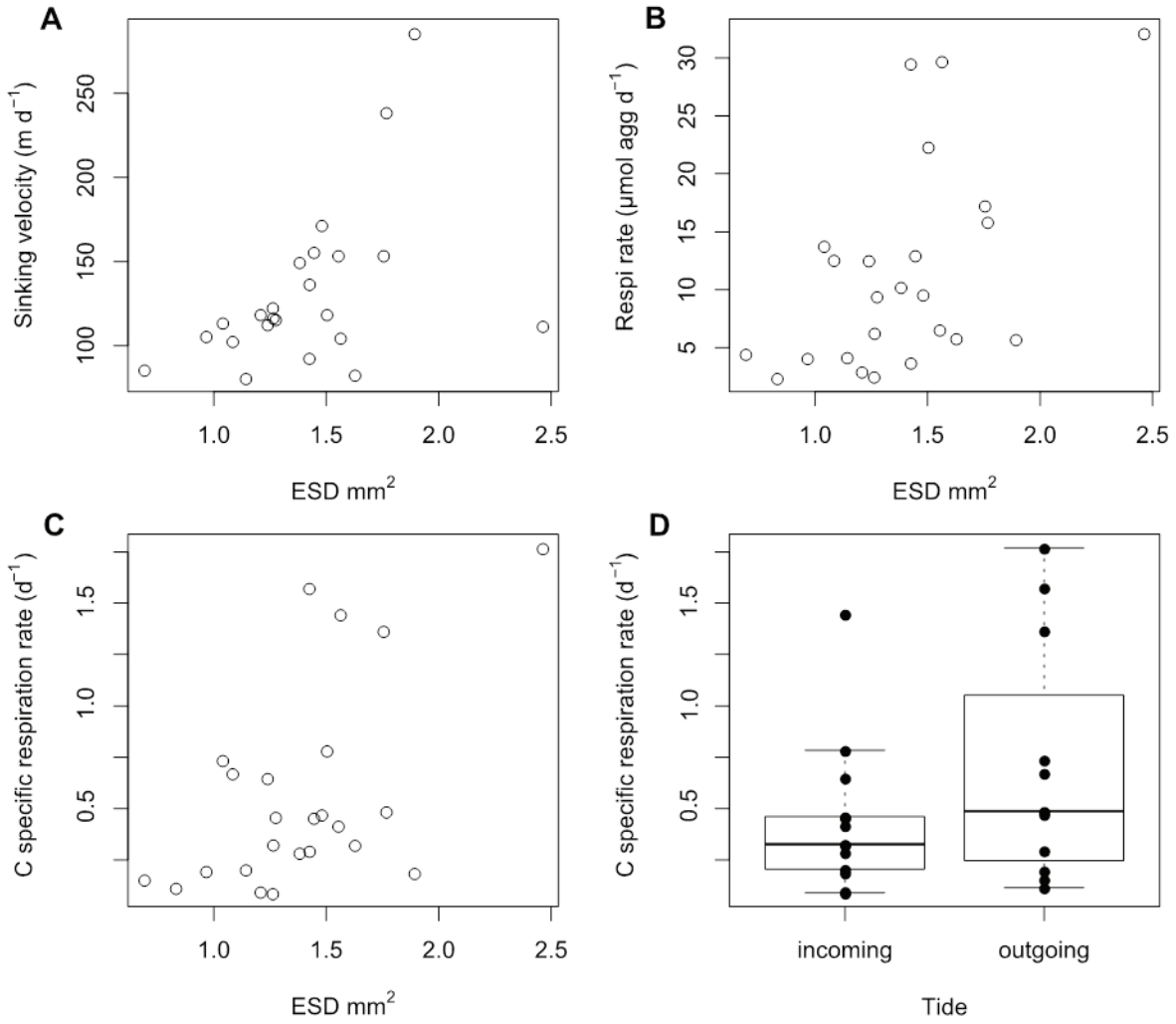

Figure 9: Sinking velocities and respiration rates of individual aggregates (A, B). Carbon specific respiration rates plotted against equivalent spherical diameter (ESD) and tide (C, D).

BCC and oxygen consumption on single aggregates

The BCC of single aggregates was in general very heterogeneous but tended to be dominated by Gammaproteobacteria, especially of the orders Vibrionales and Alteromonadales. Their dominance on individual aggregates resulted in a low Inverse Simpson index. Highest relative abundances of Vibrionales were found on all sampled aggregates at T13-O and one of the replicate aggregates sampled at T8-I and T14-O. In accordance with their presence in S and L particles, Rhodobacterales and Flavobacteriales also occurred in high relative abundances in the AG fraction. Similar to the patterns observed in the S and L fraction, Erysipelotrichales decreased in their relative abundances after T8-I. Individual aggregates were characterized by high relative abundances of Fusobacteriales. Although in general only present in very low relative abundances in the S and L fraction, Clostridiales were present with elevated relative abundances in two aggregates during the second half of the sampling period. The order Planctomycetales was only present in low relative abundances.

There seemed to be a trend towards higher C specific respiration rates from T8-I onwards (Figure 10). Although characterized by strong oxygen consumptions, outliers with C specific respiration rates above 100% were still oxic in the aggregate center. OTUs of the genera *Vibrio* and *Photobacterium* displayed the strongest correlation with C specific respiration rates.

The RDA model using respiration rate (adjusted $R^2 = 0.07$, $F_{1, 20} = 2.76$, $p = 0.004$) and tide (adjusted $R^2 = 0.03$, $F_{1, 20} = 1.71$, $p = 0.07$) as predictor variables was best suited to explain differences in BCC (RDA, AIC =187, adjusted $R^2 = 0.1$, $F_{1,21} = 1.72$, $p = 0.034$). Although from an ecological point of view, BCC rather explains respiration rate than the other way around.

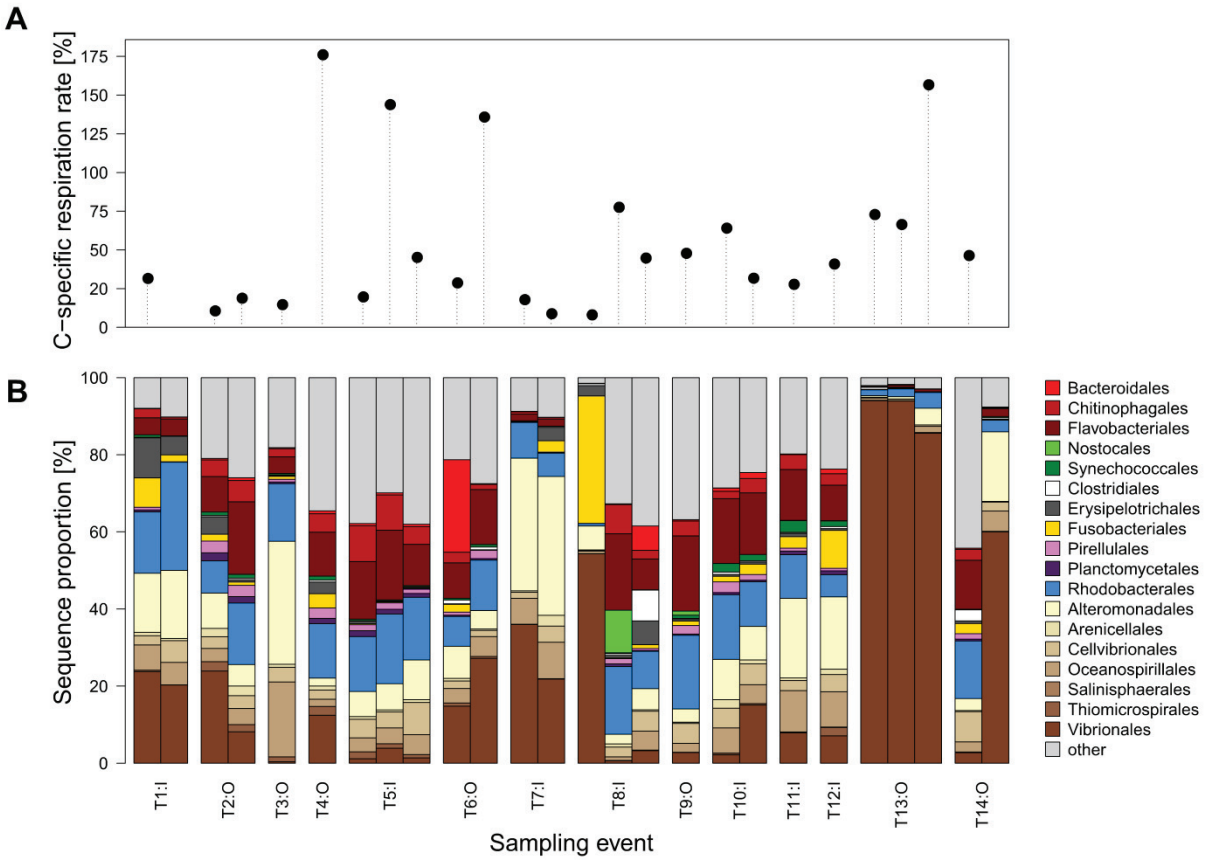

Figure 10: Carbon specific respiration rates and sequencing results of the same aggregate (there are no microsensor results for aggregate number 2 and 26. "I" and "O" indicate "incoming" and "outgoing" tides, respectively.

DISCUSSION

Excess feeding of cultured fish and their excrements as well as stimulation of phytoplankton blooms in response to increased eutrophic conditions, lead to high POM concentrations in the water column. Particles promote the horizontal and vertical connectivity of ecosystems by sinking to the sea floor and due to transportation via currents to adjacent ecosystems. Therefore, we propose that monitoring their abundances, CN content, stable isotope values and attached BCC can serve as indicators for aquaculture effects on local and adjacent coastal habitats.

This study provides the first detailed characterization of aquaculture derived particles in the Bolinao mariculture area. Our results show that GoPro action cameras can be a cost efficient and reliable tool to gather basic information about the abundance and size distribution of particles and aggregates within the water column. Although the variations in tidal amplitude affected our results, we still detected a clear signal of aquaculture influences on marine particle characteristics, which were paralleled by changes in the attached microbial community composition. Taken together the results indicate a strong environmental impact caused by aquaculture activities in the Bolinao mariculture area.

Establishment of GoPros as functional particle detection system

After the calibration of the GoPro system, it was tested with aggregates, whose sizes were correctly determined from the pictures. These test also indicated that in order for particles to appear sharp on the resulting pictures, which is a prerequisite for accurately determining their sizes, a minimum distance of 20 cm between GoPro lens and particle chamber was necessary. In the field, the GoPro-based particle detection system in its current state was able to reliably record particles with diameters between 1-8 mm. The detection of larger particles was limited due to their low concentrations in the environment. Therefore, although the system may allow the recording of larger particles, the accurate determination of their concentrations may be difficult. The lower range (<1 mm) was obstructed by optics. However, with further optimization (also by the manufacturer) the detection level might be expanded to make it possible to survey a major size range of marine snow.

Even with the limited size sensitivity, the GoPro system delivered a reliable and consistent estimate about aggregate concentrations and their size distributions in the determined size range at our field sites, which can serve as indicators of aquaculture influence.

Tidal effects on environmental parameters

Temperatures, salinities, Secchi depths and Concentrations of Chla and DO were within the range of previous results from the area (White et al. 2007).

With the exception of T6-O and T9#-O, sampling times between 1-2.5 hours before the turning point of tides were chosen. This was to ensure that during outgoing tide water masses from within the channel, i.e. most strongly influenced by the intense aquaculture activity, were transported past the sampling location during our sampling. Conversely during incoming tide, fresh sea water from the South China Sea was moving past the sampling station at the time of sampling. Therefore, in this study outgoing tide samples can be seen as a measure for aquaculture activities, whereas incoming tide samples served as a reference of less polluted water masses from the open sea. This is, however, a simplification as water movement and mixing patterns in the channel are rather complicated (Rivera 1997). Average residence times of up to 35 days in the center of the channel and of up to 5 days at our sampling site indicate that the same water masses can move back and forth for days within the channel (White et al. 2007). Additionally, seasonality, weather and tidal amplitude affect the channel conditions (White et al. 2007). All of the above can change on daily basis, making the system highly variable at both temporal and spatial scales.

During the sampling period, three distinct temporal phases (**Phase 1:** Sampling days T1-T4; **Phase 2:** Sampling days T5-T8; **Phase 3:** Sampling days T10-T14) could be determined based on the respective tidal amplitude (Figure S3). These phases showed pronounced changes in environmental parameters, such as DO, temperature, salinity, Chla and particle abundances, suggesting that neap and spring tides may affect the system in different ways. This notion could result from less or more supply of fresh sea water into the channel with neap and spring tide, respectively (White et al. 2007). However, the first and second neap tides do not carry the same environmental signatures indicating that the tidal effects are convoluted and days cannot be seen as separate entities. In contrast, they are influenced by the environmental conditions prevailing at the previous days.

Particle abundances and biogeochemistry vary with tide and over time

Despite the high variability of environmental parameters of the system, we were able to observe a clear signature of aquaculture activities. The outgoing tides tended to carry higher numbers of particles than incoming tides. Labile organic carbon content and mass fluxes tended to be higher in outgoing samples, confirming that aquaculture activities strongly

increased the POM-load in this area. Previous results from this and other areas show that aquaculture activities are associated with the accumulation of aquaculture waste products such as uneaten fish feed, fish feces and phytoplankton blooms due to the excess nutrient input (Iwama 1991; Karakassis 1994; Holmer et al. 2002; Reichardt et al. 2007, Hassenrück et al, in prep.).

High carbon specific respiration rates indicate that microbial activity was stimulated in the aquaculture area because of the eutrophic conditions, high temperatures and continuous supply of labile organic matter (D'Avanzo et al. 1996). These high aggregate attached respiration rates have the potential to contribute to water column hypoxia, which is a recurring and increasing phenomenon in the area (Reichardt et al. 2007; San Diego-McGlone et al. 2008). Additionally, high aggregate sinking velocities of 80 to 286 m d^{-1} enabled a quick POM export to the 20m deep sea floor, where the copious accumulation of organic matter leads to anoxia (Holmer et al. 2002; Reichardt et al. 2007). Anoxia in the sediments is confirmed by the observation of thick *Beggiatoa* mats on the muddy sediments in this area (Reichardt et al. 2007), a condition that has displaced the majority of the benthic macrofauna (Nacorda et al. 2012).

Stable isotopes are valuable markers for detecting the impact of aquaculture activity (Sarà et al. 2004). For instance, $\delta^{13}C$ can be used to trace the origin of organic matter (Hedges and Parker 1976; Schindler Wildhaber et al. 2012; Kopprio et al. 2014). The terrestrial sources in fish feed pellets (e.g. soy beans) may explain the more negative $\delta^{13}C$ values in pellets vs. natural aquatic POM. Additionally, phytoplankton may contribute to the fractionation of the $\delta^{13}C$ (Hinga et al. 1994). Depending on different environmental factors, such as ambient CO_2 concentrations and temperature, the cells may preferentially fix $^{12}CO_2$ molecular species (Hinga et al. 1994).

In our study, $\delta^{13}C$ values of POM differed between incoming vs. outgoing tides, rendering $\delta^{13}C$ a good proxy for aquaculture activity in the Guiguiwanen channel system. The less negative $\delta^{13}C$ values may originate from fish feces, as previously discussed (Sarà et al. 2004; Holmer et al. 2007). Nevertheless, it is also possible that the previously higher abundances of diatoms in Bolinao (White et al. 2007), which have higher $\delta^{13}C$ values (Fry et al. 1991) contribute to increased $\delta^{13}C$ values in outgoing water masses.

Although the tidal effect was not as pronounced on the $\delta^{15}N$ values, their depletion, possibly as a result of the signature of fish feed, may also be an indicator for aquaculture activities.

Aquaculture impacts on bacterial alpha diversities and community composition

The bacterial diversity (Inverse Simpson Index) of L particles tended to reflect the three phases of tidal amplitude with a time lag of about 1-2 days. This suggests that alpha diversity may be connected to the highly variable environmental parameters in the Guiguiwanen channel.

Despite the high variability connected to changes in tidal amplitude, the effect of aquaculture activities on bacterial alpha diversities was visible: While the Inverse Simpson index tended to be higher in the small (S) fraction during outgoing tide, the opposite, i.e. lower alpha diversities, was the case for large (L) particles and single aggregate (AG) samples. This could indicate a differential influence of aquaculture activities on the different size classes of particles.

The BCC of the S fraction was clearly influenced by the eutrophic conditions of the area. This was mirrored by the presence of bacteria, indicative for high nutrient and OM concentrations (e.g. Arenicellales (Teramoto et al. 2015). Additionally, higher alpha diversities might have resulted from stimulated phytoplankton growth in response to the coastal eutrophication.

In the L and AG fraction, the high relative abundances and at times dominance of bacteria, such as Erysipelotrichales and Vibrionales, decreased alpha diversities in several outgoing samples. The same orders have previously been detected in high relative abundances in milkfish guts, where bacterial Inverse Simpson indices were often below 10 (Hassenrück et al in prep.). Since microscopic analysis revealed fish feces as a major component of many aggregates in the Bolinao area, this suggests that the bacterial signature (alpha diversity and BCC) of fish feces can affect particles in Bolinao. Since fish gut bacteria were characterized by lower relative abundances in the S fraction, it can be concluded that the effect was mainly on L particles, possibly because the fecal material of milkfish was in the range of L rather than S particles. The direct link between bacteria from farmed fish and substrate-attached bacteria is further supported by the presence of other milkfish gut bacteria, such as Fusobacteriales and Clostridiales, in L and AG samples. The prevalence of Erysipelotrichia, Fusobacteriia and Clostridia in substrate-attached bacteria of previous sampling events in the area (Hassenrück in prep.) indicates that our observations were not an isolated occurrence. Rather the particle and aggregate-attached BCC, in particular the presence of gut bacteria, can be an indicator for aquaculture activities.

The fast sinking aggregates quickly reach the sea floor beneath the cages. Consequently, the rapidly sinking, large particles can act as vectors to deliver fish gut bacteria, such as Fusobacteriales, to the sediments, where they have also been shown to thrive (Moncada et al. in press). On the one hand, this highlights the role of particles in habitat connectivity. On the other hand, the preferential occurrence of milkfish gut bacteria on fast sinking L particles, could suggest that their distribution is a rather local phenomenon, which mainly affects underlying sediments.

However, slower sinking particles may also be transported horizontally. In Bolinao, it has been suggested that there is a connectivity of the aquaculture areas with adjacent sea grass beds (Ferrera et al. 2016). Furthermore, the presence of copiotrophic bacteria, including *Vibrio*, on corals close to aquaculture affected sites (Arboleda and Reichardt 2009), indicates that aquaculture derived particle can even act as vectors of potential pathogens.

Vegetated coastal habitats (including e.g. sea grass beds) provide important ecosystem functions (Barbier et al. 2011; Greiner et al. 2013). On a global scale, they even act as carbon sinks (Siikamäki et al. 2013; Ahmed et al. 2017). As world-wide, aquaculture activities are expected to expand even further to meet global food supply, it is important that this expansion will be in a more sustainable way (Ahmed et al. 2017). We cannot risk destructing more coastal ecosystems and their important services, including carbon sequestration.

CONCLUSION

This study reveals that particle abundances and characteristics can serve as indicators for aquaculture impact on the coastal marine environment. Using GoPros as particle detection system can be an easy and cost efficient way to monitor *in situ* abundances of aquaculture derived particles. In this study off Bolinao, outgoing tides transported the aquaculture impacted waters from within the Guiguiwanen channel past our sampling location. Conversely, during incoming tides less polluted waters from the South China Sea passed our sampling station.

Despite high environmental variability, particle characteristics, such as the mass of sinking particles, their POC concentrations, their $\delta^{13}C$ values, their attached bacterial alpha diversities and BCC, differed between incoming and outgoing tides. Aquaculture activities led to higher masses of sinking particles, higher POC concentrations in the particles and more positive $\delta^{13}C$ values.

On single aggregates and L particles, the aquaculture impact resulted in lower median Inverse Simpson indices, which could be linked to the at times high relative abundances of fish gut derived bacteria. Microscopic analysis confirmed the presence of fish feces in aggregates, which probably due to their sizes were preferentially incorporated into L rather than S particles. The BCC of S particles was impacted by aquaculture activities in a different way: High relative abundances of copiotrophic bacteria and efficient particle degraders reflect the eutrophic conditions.

Microbial respiration rates of the aggregate-attached microbial communities indicated a strong O_2 drawdown by bacteria, which has the potential to contribute to the observed recurring hypoxic conditions in the waters off Bolinao.

Fast sinking velocities of large single aggregates suggest that the large fraction of aquaculture derived particles sink to the shallow sea floor rapidly, leading to a rather local impact. However, as previous research in adjacent ecosystems indicates, currents can transport some of the (probably small) aquaculture impacted particles to adjacent ecosystems, where the excess nutrient, OM input and bacteria can have detrimental effects. Given the important ecosystem services of coastal habitats, it is necessary to reduce these aquaculture impacts by adopting more sustainable practices in the future.

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Conflict of Interest

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Table S1: Environmental parameters measured at 2, 5, 7 and 10 m depth.

Sampling day	Date & Time	Tide	Depth (m)	Temp °C	pH	Salinity	HDO (%)	HDO (mgL^{-1})	Chl µg L^{-1}	Turbidity	Particles L^{-1}
1	27.04.2018 08:43	incoming	2	31.74	8	34.72	76.2	4.56	3.59	0	NA
2	28.04.2018 12:50	outgoing	2	31.94	7.99	34.77	77.1	4.6	3.44	0	187
3	30.04.2018 14:46	outgoing	2	33.38	8.03	35.24	116.93	6.81	12.91	2.1	92
4	01.05.2018 15:46	outgoing	2	32.97	7.97	35.1	104.4	6.12	10.74	1.7	35
5	02.05.2018 08:23	incoming	2	32.24	7.99	34.89	77.5	4.6	8.49	0.1	50
6	03.05.2018 15:58	outgoing	2	32.81	8.11	34.81	115.93	6.83	10.66	0.4	50
7	04.05.2018 09:10	incoming	2	32.09	8	34.72	76.03	4.53	4.17	0.03	43
8	05.05.2018 09:40	incoming	2	31.57	8.12	34.48	96.9	5.83	2.1	0	27
9	07.05.2018 16:01	outgoing	2	31.18	8.06	33.52	104.25	6.34	0.98	0	33
10	08.05.2018 12:13	incoming	2	30.96	7.99	33.63	69.77	4.26	2.36	0.07	28
11	09.05.2018 12:57	incoming	2	31.42	7.99	33.71	72.27	4.38	0.74	0	111
12	10.05.2018 14:17	incoming	2	31.74	7.96	33.81	73.67	4.44	1.82	0	72
13	13.05.2018 13:11	outgoing	2	31.63	NA	33.53	101.87	6.15	1.11	0	61
14	14.05.2018 13:50	outgoing	2	NA	NA	NA	NA	NA	NA	NA	71
1	27.04.2018 08:43	incoming	5	31.84	7.96	34.85	68.2	4.07	3.81	0	46
2	28.04.2018 12:50	outgoing	5	31.83	7.95	34.82	68.1	4.07	3.65	0	99
3	30.04.2018 14:46	outgoing	5	32.84	7.88	35.18	62.67	3.69	7.43	0.57	161
4	01.05.2018 15:46	outgoing	5	32.3	7.81	35.03	44.57	2.64	5.61	0.93	5
5	02.05.2018 08:23	incoming	5	32.15	7.92	34.91	58.4	3.47	7.65	0	10
6	03.05.2018 15:58	outgoing	5	31.89	8	34.75	70.47	4.21	8.64	0	25
7	04.05.2018 09:10	incoming	5	31.57	7.93	34.66	46.57	2.8	3.64	0	43
8	05.05.2018 09:40	incoming	5	31.44	8.11	34.49	87.8	5.29	2.39	0	42
9	07.05.2018 16:01	outgoing	5	31.16	8.08	33.6	103.9	6.32	0.83	0	43
10	08.05.2018 12:13	incoming	5	30.92	8.03	33.63	77.1	4.71	0.87	0	24
11	09.05.2018 12:57	incoming	5	31.32	7.98	33.71	70.35	4.27	0.77	0	116
12	10.05.2018 14:17	incoming	5	31.47	7.94	33.85	67.87	4.1	1.01	0	30
13	13.05.2018 13:11	outgoing	5	31.41	NA	33.56	92.35	5.6	0.91	0	53
14	14.05.2018 13:50	outgoing	5	NA	NA	NA	NA	NA	NA	NA	58
1	27.04.2018 08:43	incoming	7	32.39	7.7	35.35	35.67	2.11	4.81	0.47	NA
2	28.04.2018 12:50	outgoing	7	31.92	7.91	34.92	55.97	3.34	3.87	0	172
3	30.04.2018 14:46	outgoing	7	32.57	7.81	35.16	42.27	2.49	5.11	0.27	157
4	01.05.2018 15:46	outgoing	7	32.34	7.8	35.08	38.97	2.31	5.08	1.57	8
5	02.05.2018 08:23	incoming	7	31.81	7.89	34.87	43.33	2.59	4.33	0.03	17
6	03.05.2018 15:58	outgoing	7	31.44	7.96	34.61	49.83	3	5.08	0.17	20
7	04.05.2018 09:10	incoming	7	30.87	7.96	34.49	41.03	2.49	2.03	0	28
8	05.05.2018 09:40	incoming	7	31.34	8.09	34.45	82.27	4.97	2.45	0	72
9	07.05.2018 16:01	outgoing	7	30.64	8.09	33.65	104.65	6.42	0.7	0	34
10	08.05.2018 12:13	incoming	7	30.84	8.04	33.65	84.45	5.16	0.72	0	17
11	09.05.2018 12:57	incoming	7	31.33	7.92	33.82	49.9	3.02	0.5	0	106
12	10.05.2018 14:17	incoming	7	31.5	7.88	33.91	50.5	3.05	1.04	0	23
13	13.05.2018 13:11	outgoing	7	31.33	NA	33.54	99.6	6.05	2.04	0	42
14	14.05.2018 13:50	outgoing	7	NA	NA	NA	NA	NA	NA	NA	49
1	27.04.2018 08:43	incoming	10	32.31	7.71	35.45	29.57	1.75	5.12	2.3	100
2	28.04.2018 12:50	outgoing	10	32.11	7.8	35.09	38.87	2.31	3.4	0.07	90
3	30.04.2018 14:46	outgoing	10	32.29	7.76	35.12	27.2	1.61	3.37	0.32	53
4	01.05.2018 15:46	outgoing	10	31.62	7.75	34.9	17.67	1.06	2.43	0.33	9
5	02.05.2018 08:23	incoming	10	30.88	7.92	34.61	35.47	2.15	1.29	0	21
6	03.05.2018 15:58	outgoing	10	30.68	7.95	34.27	38.5	2.35	1.89	0	20
7	04.05.2018 09:10	incoming	10	30.31	8.02	34.36	48.17	2.96	1.52	1.32	34
8	05.05.2018 09:40	incoming	10	31.01	8.08	34.43	78.63	4.77	2.98	0.2	24
9	07.05.2018 16:01	outgoing	10	30.61	8.06	33.68	90.87	5.58	0.64	0	24
10	08.05.2018 12:13	incoming	10	30.94	7.94	33.83	59.83	3.65	0.49	0	21
11	09.05.2018 12:57	incoming	10	31.49	7.79	34.24	34.93	2.11	0.7	0	24
12	10.05.2018 14:17	incoming	10	31.58	7.79	34.16	31.37	1.89	0.73	0	51
13	13.05.2018 13:11	outgoing	10	31.07	NA	33.55	96.05	5.86	2.57	0	69
14	14.05.2018 13:50	outgoing	10	NA	NA	NA	NA	NA	NA	NA	51

Table S2: Microsensor results where "Replicates" are the replicate O_2 measurements of the same aggregate that led to the average respiration rate ("Average"). Using the ellipsoid surface area and the carbon content of the M particles (see bulk measurements) of the same day, respiration rates and carbon specific respiration rates ("C r rate") were calculated.

Aggregate	Date	AG	Tide	Replicates	Average (nnol cm2 h-1	Surface area (cm2)	Corg (ug mg-1)	R rate (umol agg*d-1)	C r rate (d-1)	C r rate (%)
1	27.04.2018	1	incoming	2	40.68	0.5	9.01	5.85	0.65	64.9
3	28.04.2018	1	outgoing	2	92.69	0.09	10.51	2.42	0.23	22.99
4	28.04.2018	2	outgoing	3	119.78	0.12	10.51	4.14	0.39	39.41
5	30.04.2018	1	outgoing	2	201.37	0.08	14.47	4.5	0.31	31.11
6	01.05.2018	1	outgoing	5	142.89	0.78	9.09	32.16	3.54	353.9
7	02.05.2018	1	incoming	4	85.62	0.17	10.27	4.22	0.41	41.04
8	02.05.2018	2	incoming	3	327.81	0.32	10.27	29.74	2.89	289.48
9	02.05.2018	3	incoming	2	157.33	0.21	10.27	9.46	0.92	92.11
10	03.05.2018	1	outgoing	3	50.14	0.26	6.33	3.74	0.59	59.16
11	03.05.2018	2	outgoing	4	139.55	0.43	6.33	17.3	2.73	273.38
12	04.05.2018	1	incoming	6	42.69	0.47	15.38	5.77	0.38	37.53
13	04.05.2018	2	incoming	2	53.5	0.19	15.38	2.98	0.19	19.36
14	05.05.2018	1	incoming	3	42.52	0.21	14.25	2.55	0.18	17.88
15	05.05.2018	2	incoming	3	268.91	0.29	14.25	22.35	1.57	156.86
16	05.05.2018	3	incoming	3	169.5	0.27	14.25	13	0.91	91.24
17	07.05.2018	1	outgoing	3	134.84	0.41	16.3	15.88	0.97	97.41
18	08.05.2018	1	incoming	7	223.48	0.2	9.68	12.57	1.3	129.91
19	08.05.2018	2	incoming	2	102.6	0.21	9.68	6.31	0.65	65.22
20	09.05.2018	1	incoming	2	142.37	0.25	17.92	10.27	0.57	57.32
21	10.05.2018	1	incoming	2	74.91	0.31	7.9	6.6	0.84	83.57
22	13.05.2018	1	outgoing	2	248.12	0.19	9.37	13.83	1.47	147.49
23	13.05.2018	2	outgoing	3	274.33	0.16	9.37	12.62	1.35	134.58
24	13.05.2018	3	outgoing	3	396.49	0.26	9.37	29.54	3.15	315.1
25	14.05.2018	1	outgoing	2	118.75	0.28	10.17	9.62	0.95	94.53

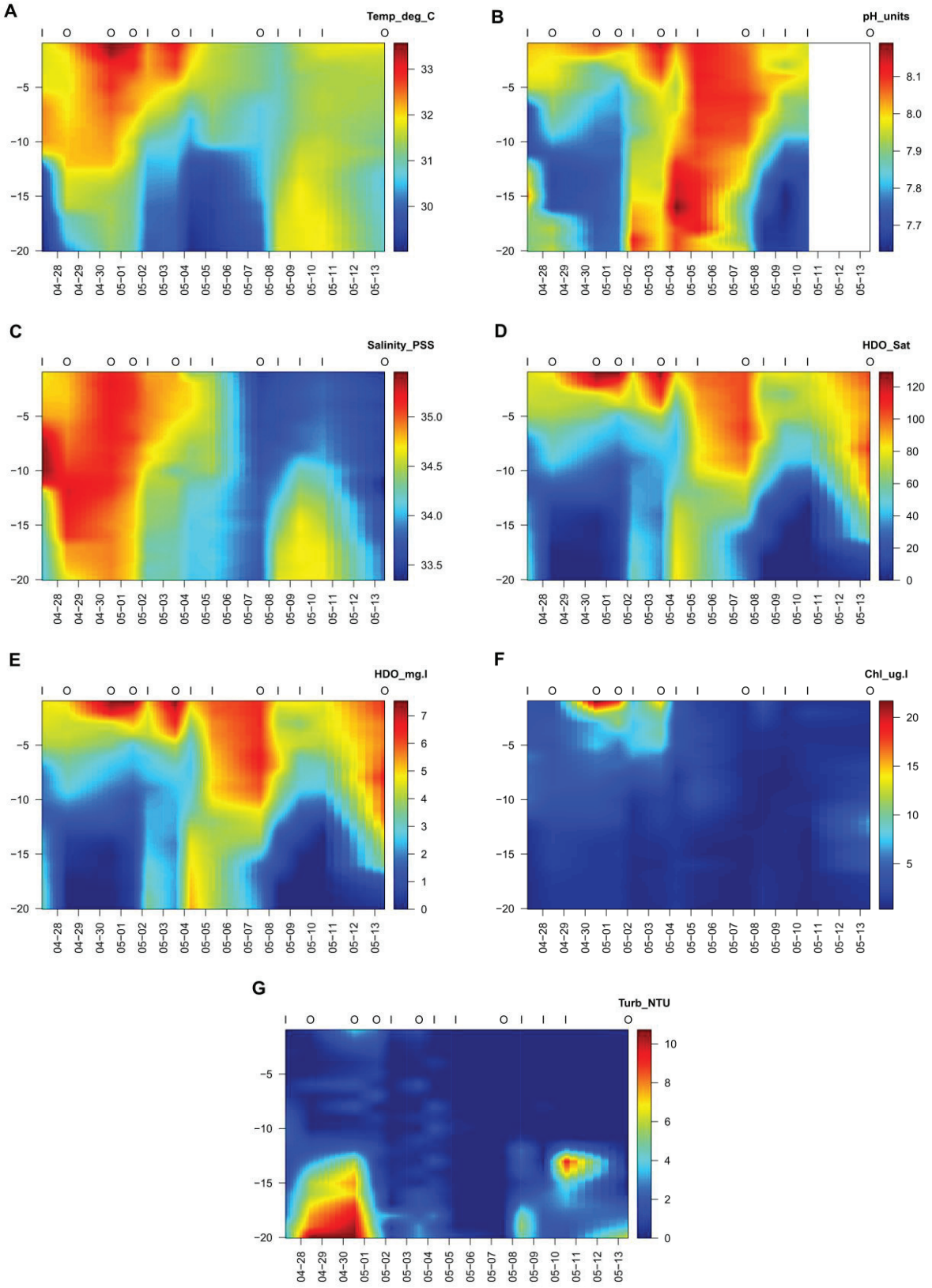


Figure S1: Changes in environmental parameters (temperature (A), pH (B), salinity (C), dissolved O_2 (D), chlorophyll *a* (E) and turbidity(F)) during the sampling period. Top axis indicates the sampling events (T1:T14) and incoming (I) vs outgoing (O) tide.

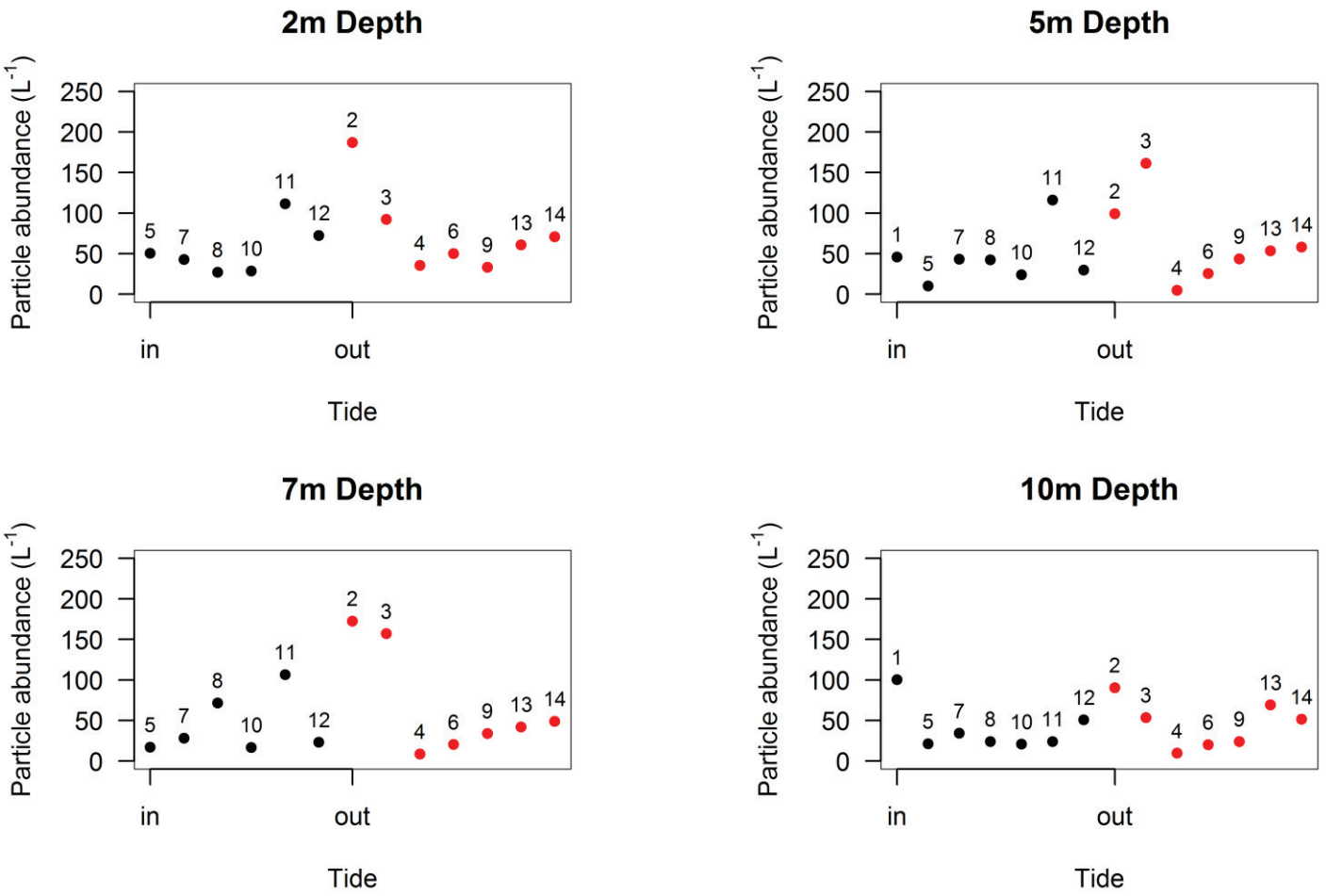


Figure S2: Changes of total particle abundances over time with numbers indicating sampling days (T1-T14).

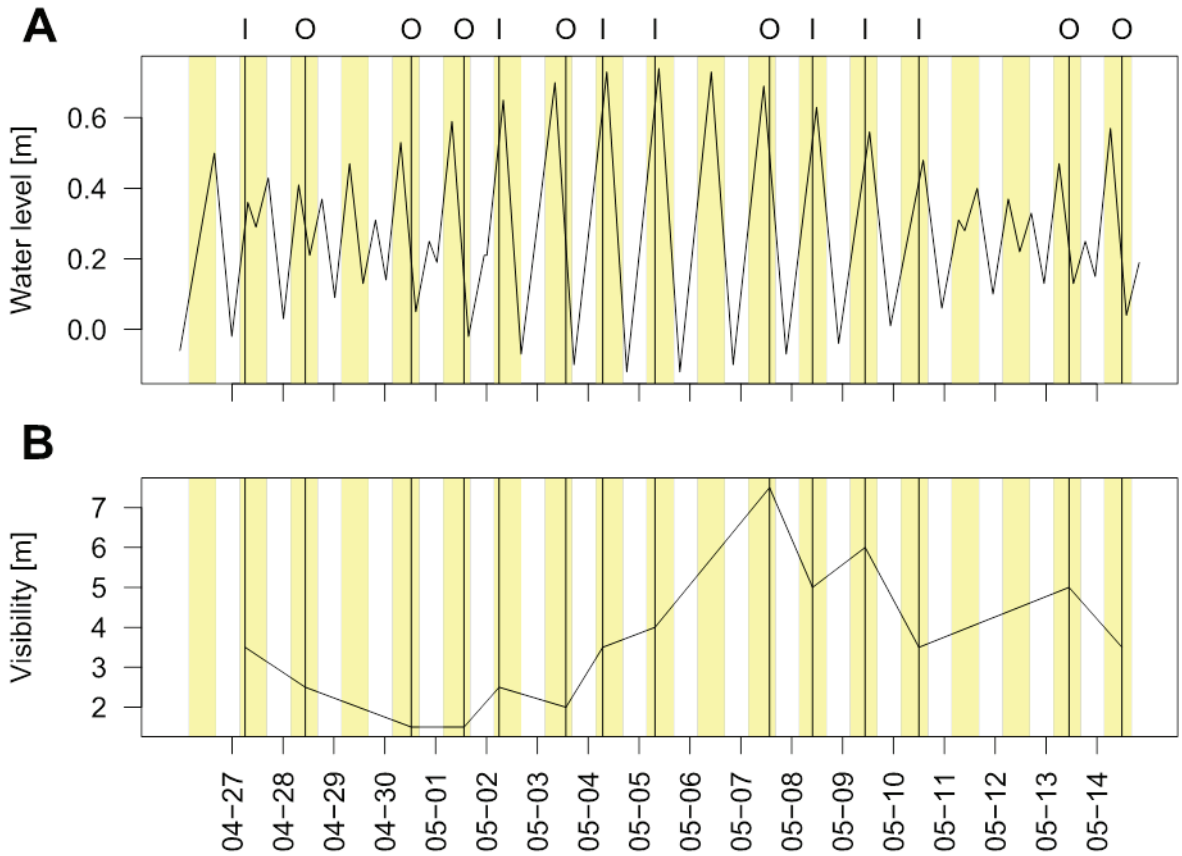
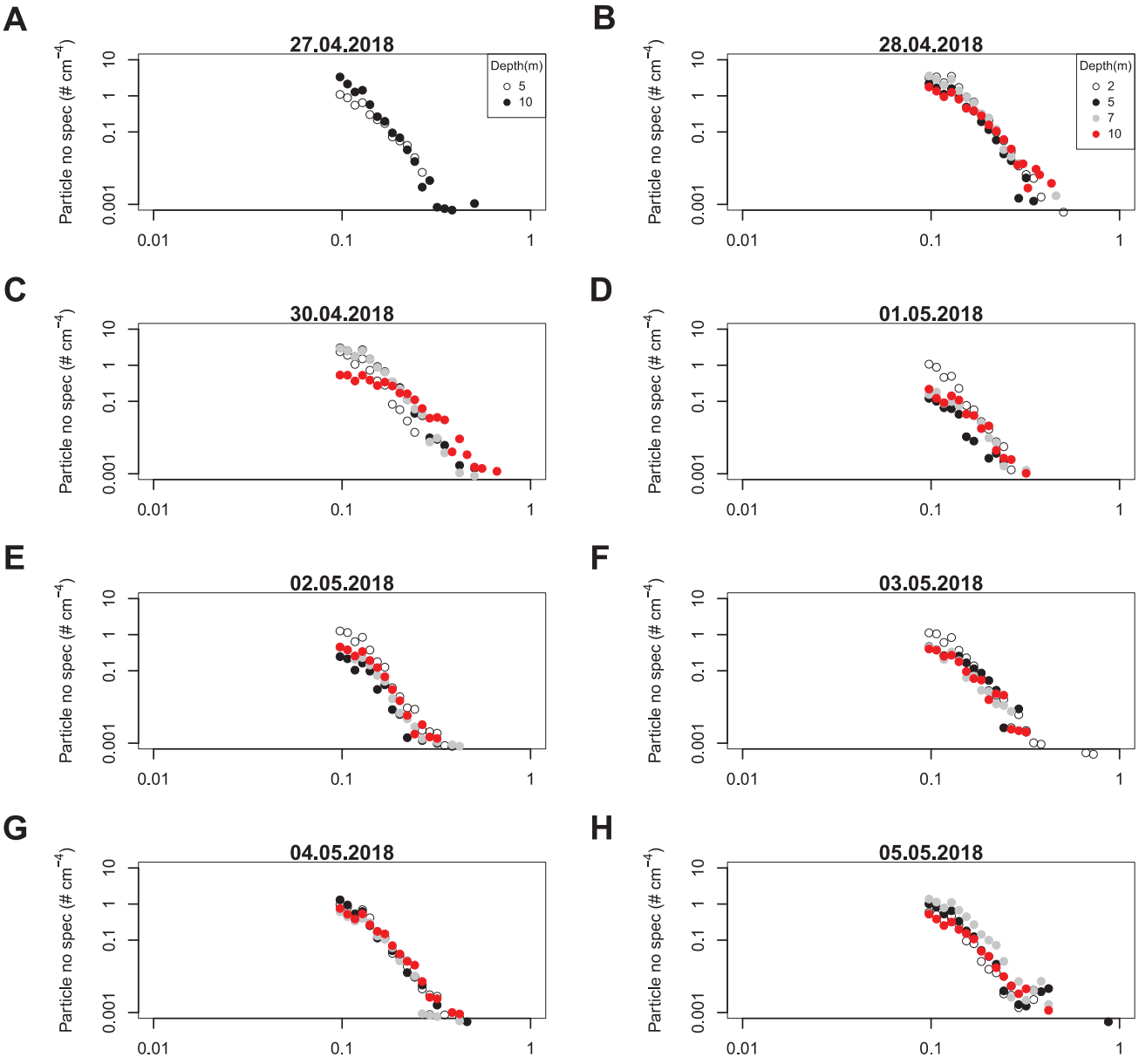


Figure S3: Changes in water level (A) and Secchi depth (B) during the sampling campaign. In the top panel, I and O indicate sampling at incoming and outgoing tide, respectively.


A
27.04.2018
B
28.04.2018
C
30.04.2018
D
01.05.2018
E
02.05.2018
F
03.05.2018
G
04.05.2018
H
05.05.2018
Particle no spec (# cm^{-4})
0.001
0.1
1
10
0.01
0.1
1
Depth(m)
5
10
Depth(m)
2
5
7
10

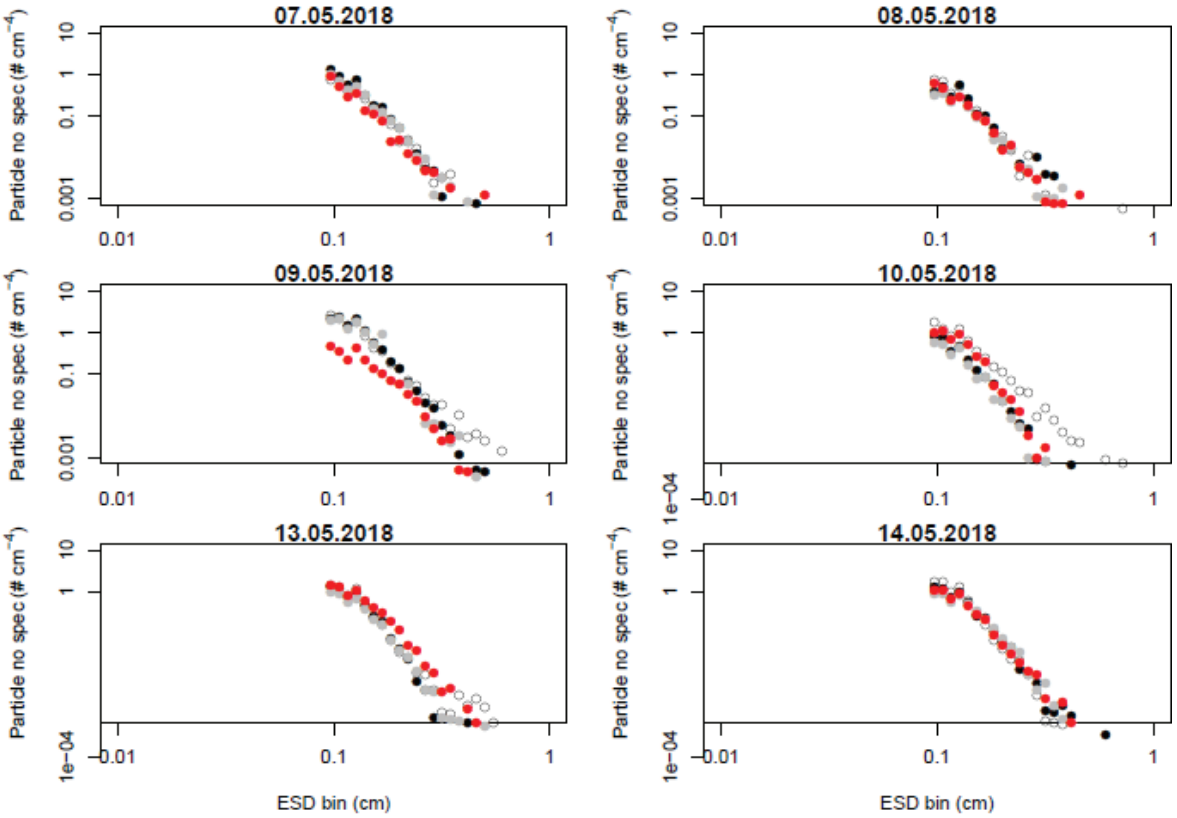
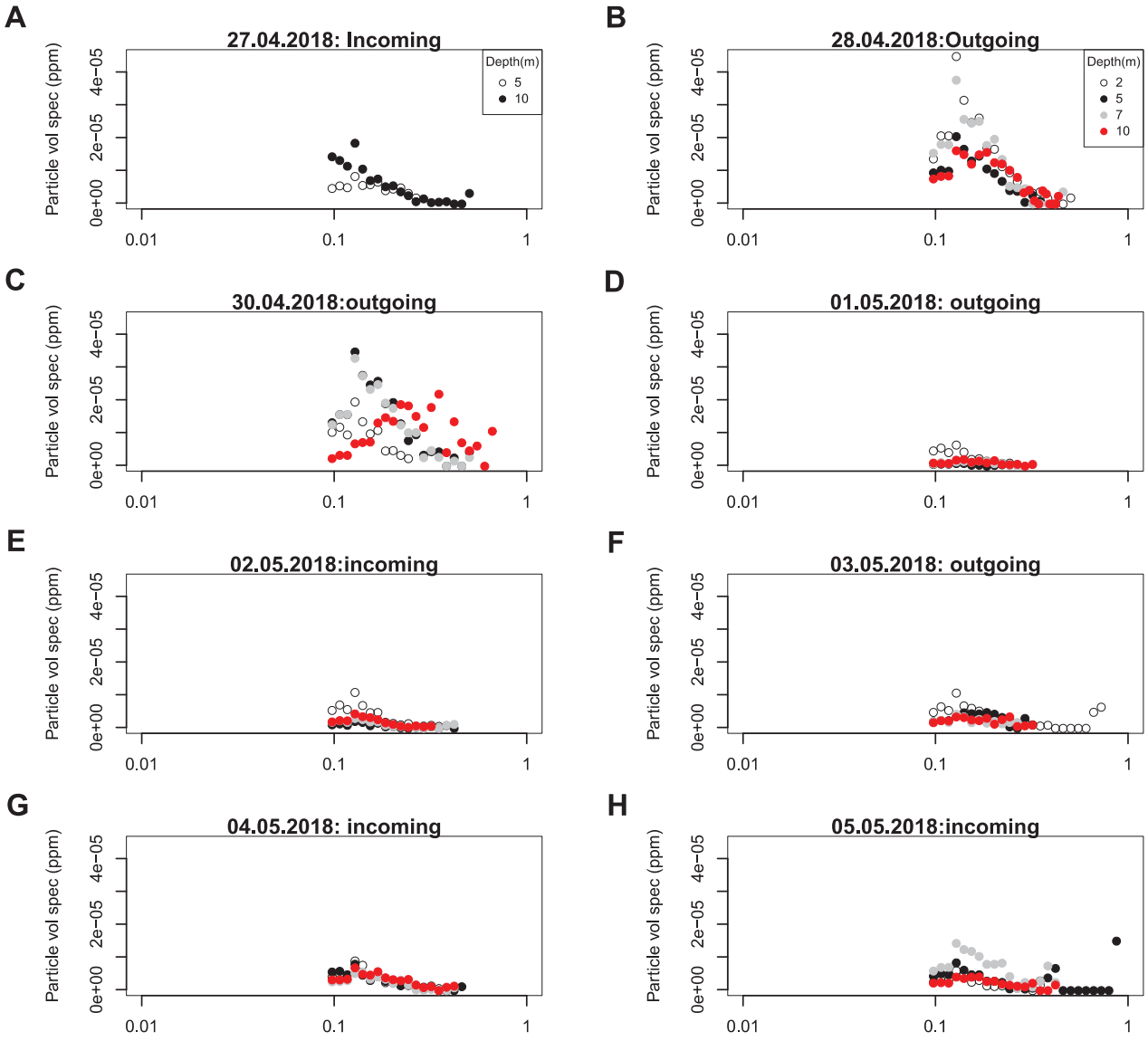


Figure S4: Log particle number spectra plotted against log ESD bins.


A
27.04.2018: Incoming
Depth(m)
5
10
B
28.04.2018:Outgoing
Depth(m)
2
5
7
10
C
30.04.2018:outgoing
D
01.05.2018: outgoing
E
02.05.2018:incoming
F
03.05.2018: outgoing
G
04.05.2018: incoming
H
05.05.2018:incoming
Particle vol spec (ppm)
0e+00
2e−05
4e−05
0.01
0.1
1

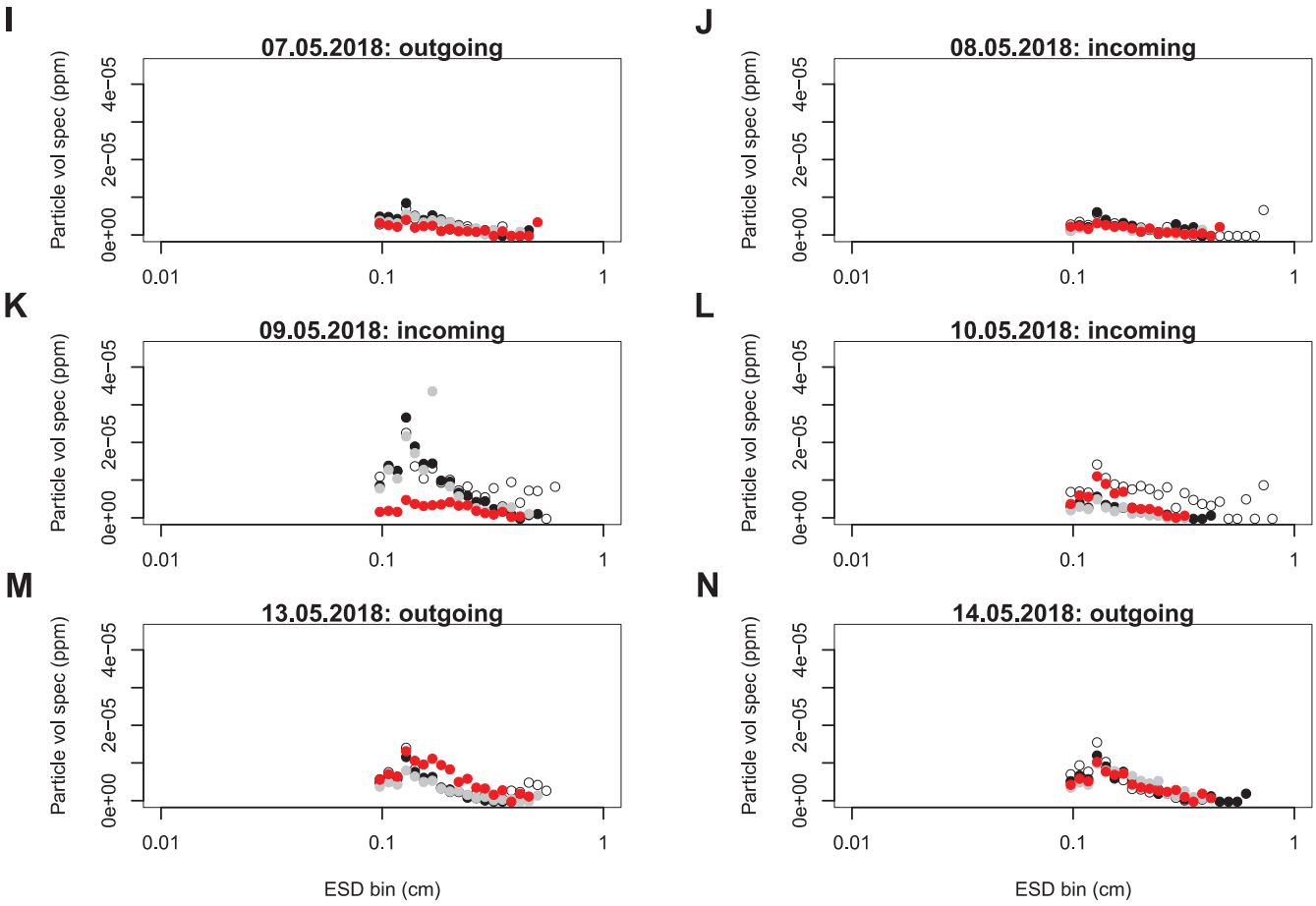


Figure S5: Particle volume spectra in parts per million (ppm) plotted against log ESD bins.

Table S 3: The CN ratio of bulk particulate organic matter

Date	Timepoint	Tide	C:N Ratio
27.04.2018	T1	in	8.73
28.04.2018	T2	out	6.37
30.04.2018	T3	out	6.03
01.05.2018	T4	out	6.83
02.05.2018	T5	in	5.22
03.05.2018	T6	out	8.59
04.05.2018	T7	in	14.45
05.05.2018	T8	in	8.21
07.05.2018	T9	out	5.74
08.05.2018	T10	in	7.67
09.05.2018	T11	in	10.6
10.05.2018	T12	in	7.88
13.05.2018	T13	out	14.24
14.05.2018	T14	out	10.05

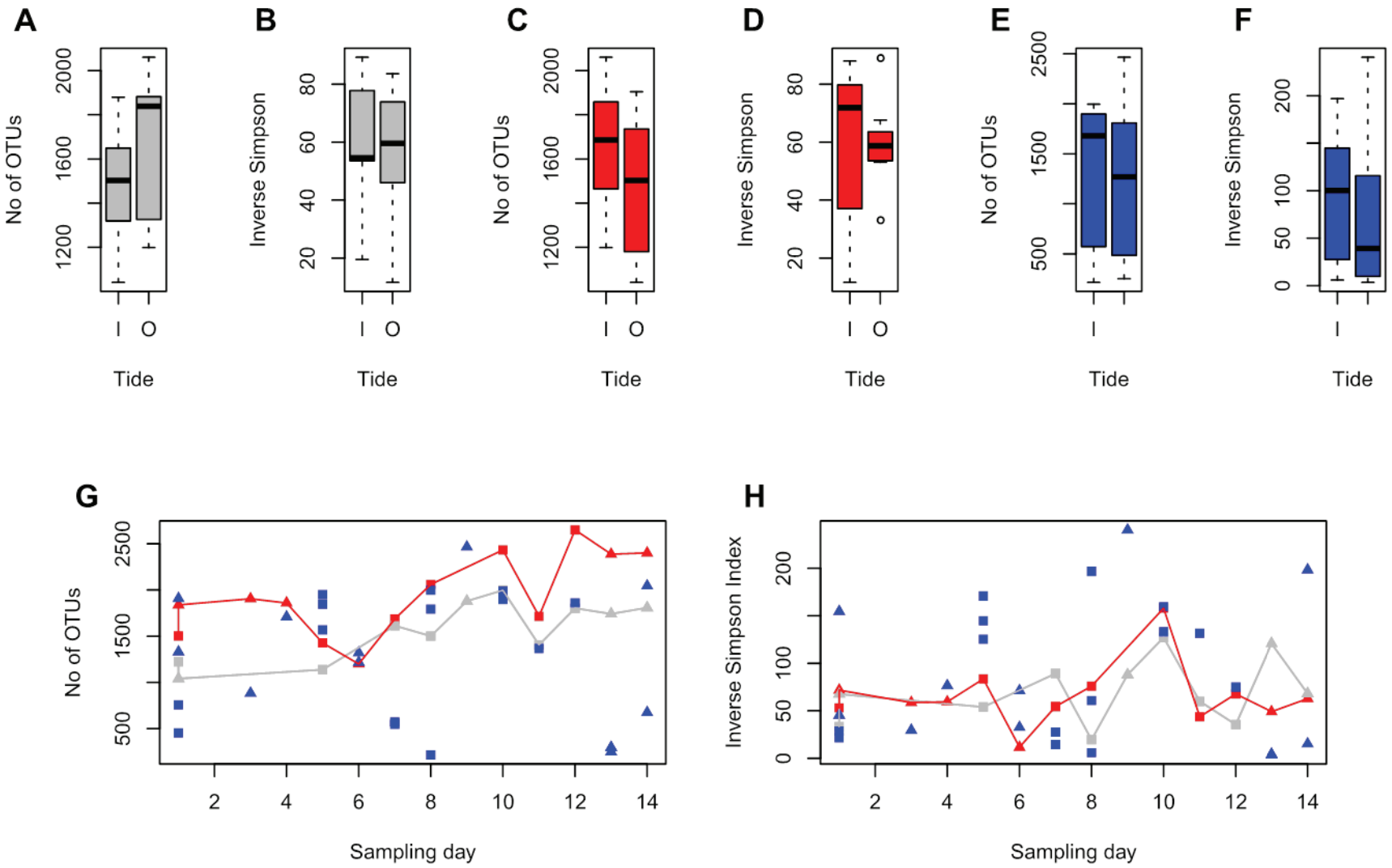


Figure S6: Variations in bacterial alpha diversity of small (S) and large (L) particle fractions as well as single aggregates (AG) with tide displayed as number of OTUs and Inverse Simpson index (A-F). The temporal trend of alpha diversities in all fractions is shown in G and H.

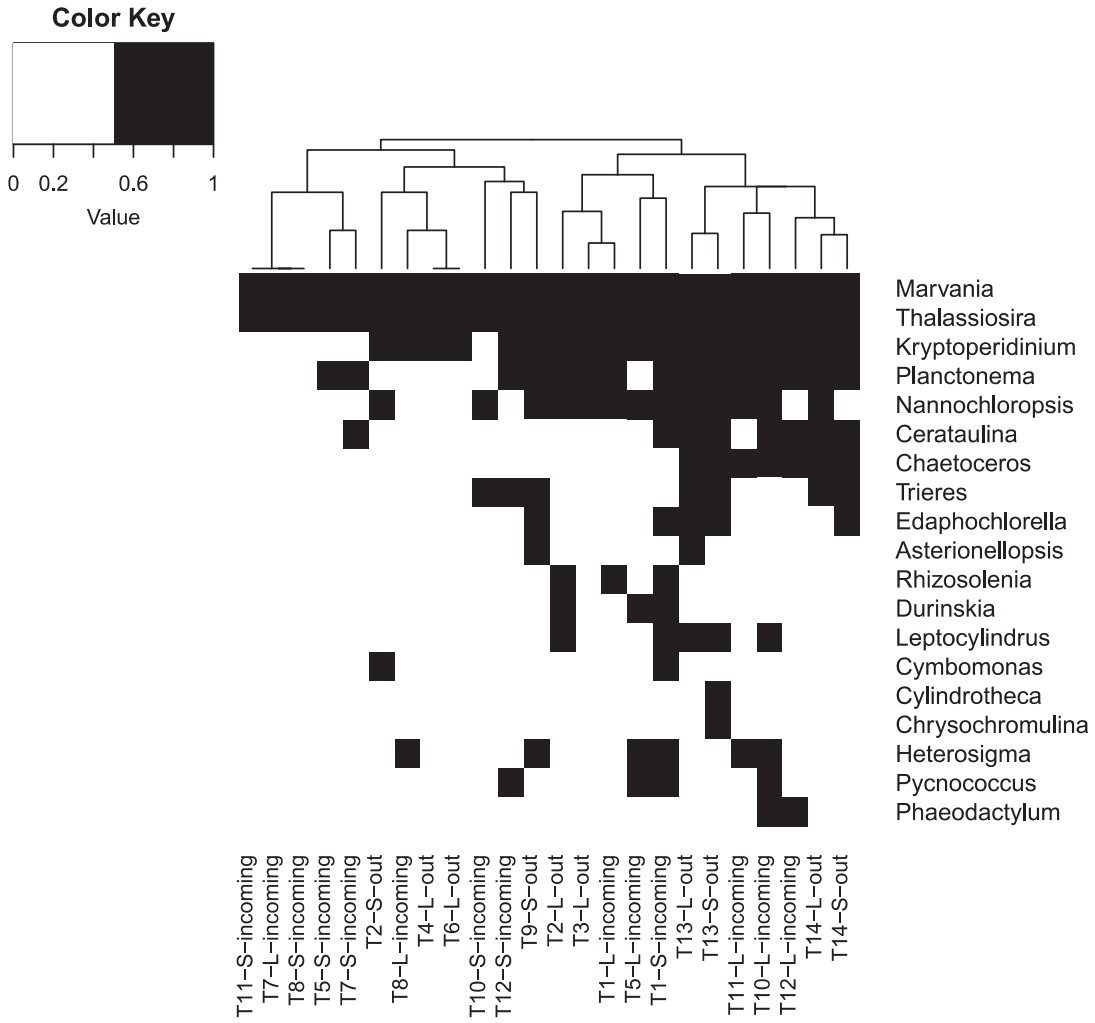


Figure S7: Presence/ absence of Chloroplast sequences. There is a trend towards higher diversity towards the end of the sampling campaign. Note For T3-S, T4-S and T6-S as well as for T9-L there are no chloroplast data because these are the samples, which will be resequenced.

DNA-Extraction Protocol

Protocol DNA extraction from size fractionated and single aggregate samples: Modifications based on (Bossier et al. 2004; Nercessian et al. 2005; Wang and Wang 2012)

1a. Thaw samples on ice if they were stored at -20/ -80°C

1b. Put filter in 2 ml bead tube filled beforehand

For single aggregate (low yield) samples: 1pc of 3 mm beads, 10-20 pcs of 1mm beads,50-100 mg of 0.1 mm beads

For filters (high yield) samples: 0.25 mg of 1 mm beads, 0.3mg of 0.1mm beads, 3 pc of 3 mm beads

2. Add 500 µl CTAB-buffer (2x conc.)

3. Add 25 µl of 20% SDS and 50 µl 10% N-Lauroylsarcosin (both pre-heated at 60°C to dissolve properly)

4. Place the Cryotubes into the FastPrep for 20sec, setting 4 m/s

5. To reduce the foam and make space for the phenol briefly centrifuge the tubes at 10 000 x g (for 30s at RT)

6a. For protein digestion, add 8 µl Proteinase K (20mg/ml) and 3 µl Lysozyme (10mg/ml), mix thoroughly (no vortex) and incubate in heating block 65°C for 20 mins (400rpm). To ensure best mixing, invert/mix occasionally by hand (e.g. every 5min)

6b. Enzyme activity: add 3µl Lysozyme (5mg/ml) mix thoroughly, incubate at 37°C for 15 min, then add 8µl Proteinase K (20mg/ml) and incubate at in heating block 60°C for 20 mins (400rpm).

7. Under fume hood: Add 500 µl Phenol-Chloroform-Isoamylalcohol (25:24:1), mix beforehand (the volume of the bead tube should be sufficient, since the filter shouldn't take up that much space → avoid transferring the supernatant to a new tube prior to PCI addition to avoid loss of material)

8. Vortex for 2 x 30 seconds

9. Centrifuge at 16 000 x g at 4°C for 10 min

10. Pipet upper (aqueous) phase in fresh Eppendorf tube in to 2 ml tubes. Be very careful to not touch the organic phase! → if more than 600µL: 2x 400µL

11. Add 2 volumes PEG/NaCl and 2 µl vivid stain (Roboklon ≅ coloured glycogen), mix thoroughly by inverting

12. Incubate in fridge (4°C) for 2 h

13. Centrifuge at 17 000 x g at 4°C for 60 min

14. Discard upper phase by decanting, → here speed is important: best decant right after removing tube from centrifuge

15. Wash pellet with 800µl ice-cold 70% Ethanol

16. Centrifuge at 17 000 x g for 10 min

17. Discard upper phase by decanting → no rack required, put tubes directly back in the centrifuge

18. Short centrifugation step to collect remaining EtOH at bottom of tube

19. Remove all remaining ethanol by pipetting

20. Let pellet air dry (open tubes under fume hood) so REALLY all the Ethanol is evaporated (don't dry to long, max. 10 min)

21. Elute the pellet in TE buffer (adjust volume based on expected concentration). For low volumes → add directly to pellet

22. Flick tubes several times and spin down, then incubate in heating block for approximately 10 mins with 37°C and 400 rpm.

Buffers (made with DEPC-treated water)

1. CTAB-Extraction buffer
   CTAB lysis buffer BioChemica (AppliChem, Germany)

2. PEG/NaCl
   - 1,6 M NaCl (M = 58,44 g/mol) = 18,7 g NaCl
   - 30% PEG 6000 = 60 g PEG
   - Fill up with 200 ml DEPC-water
     - takes a while to dissolve --> 60°C drying oven helps

3. SDS (20%)
   Molare Masse (M) 288,38 g/mol
   For 50ml in Falcon tube: 10g SDS + DEPC water → fill up to 50ml, dissolve at 60°C in drying oven

4. N-Lauroylsarcosin (10%)
   Molar mass (M) 293.39 g/mol
   For 50ml in Falcon tube: 5g N-Lauroylsarcosin + DEPC water → fill up to 50ml, dissolve at 60°C in drying oven

5. Phenol-Chloroform-Isoamylalcohol (25:24:1)
   For 300ml: 150ml Phenol (equilibrated, pH 8.0) + 144ml Chloroform (Trichlormethane) + 6 ml Isoamylalcohol

CHAPTER V

GENERAL DISCUSSION

In this thesis the source, role and composition as well as microbial interactions within particles and aggregates (from here on particles) were determined in two very contrasting marine environments. The studies of **Chapters II and III** were conducted in waters influenced by the Eastern Boundary Current (EBC) off the coast of Mauritania/ Senegal. The study in **Chapter IV** was carried out in an aquaculture system off the coast of the Philippines. Since it is rather difficult to compare the two systems directly, the results of this thesis will be synthesized with respect to the role of particulate organic matter as a) vector for carbon export and b) indicator for aquaculture activity, separately at first. **Chapters II and III** are put into the perspective of the aims of the M129 cruise and are contrasted with results obtained in other EBC systems. **Chapter IV** is discussed in the context of the ACUTE project and other eutrophic coastal ecosystems. Since the results of this thesis indicate that the particle-attached bacterial communities play a major role in both, determining the carbon export efficiency and colonization/ degradation of aquaculture derived POM, discussions will always focus on the microbial perspective. This will culminate in a final excurse on the role of particles as hotspots, refuges and micro-islands for bacteria in eutrophic vs. oligotrophic marine systems, where results of **Chapters II, II** and **IV** will be discussed together.

Particulate organic matter as vector for horizontal and vertical carbon export in EBCs

The four Eastern Boundary currents (EBC), which are located off Mauritania (Canary Current), Namibia (Benguela Current), USA (California Current) and Peru (Humboldt Current), are among the most productive marine systems globally (Carr 2001). After the Benguela Current, the Canary Current system off the coast of North West Africa, is the second most productive EBC. EBCs account for around 10% of the global primary productivity (Behrenfeld and Falkowski 1997), having a considerable impact on global carbon export and fisheries (Carr and Kearns 2003; Lachkar and Gruber 2012 and references therein).

In EBCs, the photosynthetically-fixed carbon is exported vertically into deeper water layers or the sea floor and horizontally into the open ocean: Horizontal carbon export can be driven by offshore cyclonic and anticyclonic eddies or offshore Ekman transport (Arístegui et al. 2004), while the creation of particles (such as marine snow and fecal pellets) with a higher density than sea water, promotes its vertical export, thereby forming the biological carbon pump (BCP).

Microbial degradation of vertically exported particulate organic matter

Due to the high productivity, the BCP in EBCs has received substantial attention (Bory and Newton 2000; Bory et al. 2001, 2002; Romero et al. 2002; González et al. 2007; Fischer et al. 2009, 2016; Iversen et al. 2010; Stukel et al. 2011; van der Jagt et al. 2018). Already early studies on the role of prokaryotic microbes in EBC upwelling systems showed a significant correlation between primary and bacterial productivity (e.g. Painting et al. 1993) suggesting their role in the organic carbon degradation. More recent studies have shed further light on the importance of bacteria in carbon cycling of EBCs (e.g. Troncoso et al. 2003; Cuevas and Morales 2006a; Iversen et al. 2010).

As shown in this thesis, off Mauritania and Senegal, microbial respiration rates and bacterial successions on marine particles suggest that bacteria may affect BCP efficiency. This in line with previous studies, which found microbial carbon degradation, as estimated by respiration rates, to be around 0.1-0.2 d^{-1} (Iversen et al. 2010). In **Chapter III** we reported a shift in BCC, connected to the simulated sinking of particles through different water masses. There is one study, which has previously observed a similar shift and connected it to reduced respiration rates (Grossart and Ploug 2000). This indicates that this shift in BCC may affect the carbon degradation efficiencies of the attached bacteria, highlighting the implication of microscale changes for large scale ecosystem processes. Especially, since temperatures are expected to rise due to global warming such mechanisms could become increasingly important. Since carbon degradation rates are influenced by temperature (Iversen and Ploug 2013), which could reduce carbon export efficiencies, it would be very relevant to investigate if the observed shifts in BCC could be a mechanism to increase vertical carbon export. To shed more light on this possibility, future studies should combine respiration measurements and analysis of bacterial16S rRNA genes of microbes from the same aggregates, exposed to deep water masses

Previous studies have indeed shown that BCC is one of the main factors influencing the "mode and tempo", of organic matter degradation and hence carbon export efficiency (Enke et al. 2018). Apart from a CARD-FISH based study on PA bacteria (Thiele et al. 2015), detailed information on the FL and PA BCC off Mauritania and Senegal (and in other EBCs) was lacking. Therefore, **Chapter II** provides the first detailed description of the FL and PA BCC in the Mauritania/ Senegalese sub-region of the Canary Current system. We found high relative abundances of potential microbial key players, including Planctomycetes, Bacteroidetes and Verrucomicrobia, which are known for their substrate-attached lifestyles

and have been shown to contribute to biopolymer degradation (Delong et al. 1993; Crump et al. 1999; Woebken et al. 2007; Martinez-Garcia et al. 2012; Teeling et al. 2012; Williams et al. 2012; Mohit et al. 2014; Yung et al. 2016). Future studies using e.g. whole genome shotgun metagenomics and/ or transcriptomics could provide further evidence for a direct link between the abundances of these bacterial groups and their role in carbon turnover.

Microbial communities and processes have also received attention in other EBCs. They include (but are not limited to) studies on the bacterial zonation in result to the aging of upwelling water (Bergen et al. 2015), the BCC (including the PA fraction) in oxygen minimum zones below EBCs (Kuypers et al. 2005; Hamersley et al. 2007; Woebken et al. 2007), grazing of bacteria (Cuevas and Morales 2006) and bacterial production (Cuevas et al. 2004). However, despite the tight coupling of bacterial and primary productivity and the key role bacteria can play in the BCP, few studies have systematically investigated the different roles of FL and PA BCC in carbon degradation in other EBCs. Since FL and PA bacteria do not only differ in life-styles but also functionality (Lyons and Dobbs 2012), the systematic distinction of FL and PA bacteria in EBCs can contribute to a better understanding of their respective roles in the BCP in the globally relevant EBC systems. Here, **Chapters II** and **III** can serve as an important basis for future studies.

The role of bacteria in the food web off Mauritania and Senegal

While vertical export removes nutrients and organic matter from the euphotic zone, horizontal transportation and retention of POM supports heterotrophic processes and the marine food web in the surface ocean (Arístegui et al. 2004; Lachkar and Gruber 2012). Therefore, microbial processes investigated in this thesis are not only relevant for the vertical export of organic carbon but also in the context of food web structure, which was one of the main aims of the M129 cruise.

In EBCs, which together represent an area that constitutes <1% of the global ocean, around 20% of the global fish take is acquired (Chavez and Messié 2009). In these ecosystems productivity is determined by bottom-up processes (Chavez and Messié 2009).

Off Mauritania, upwelling leads to high primary productivity (Lachkar and Gruber 2012). Phytoplankton communities were characterized by high abundances of coccolithophores and diatoms **(Chapter II)**. Cyanobacteria were also highly abundant, indicating that prokaryotic cells contribute to the primary productivity in the area. Additionally, microscopic analysis of the phytoplankton samples revealed the presence of heterocysts (especially in the nutrient-

poor waters off Senegal) in some cyanobacterial groups, suggesting they may also perform N_2 fixation, thereby further fueling the food web from the bottom up.

Indeed, primary productivity can either be sustained by the supply of fresh inorganic nutrients (especially nitrate), e.g. due to upwelling of waters from below the thermocline, or by remineralization of OM by heterotrophic bacteria (Auger et al. 2016). This relationship between "new" vs. "remineralized" production is commonly expressed as *f-ratio* (Eppley and Peterson 1979). We have shown that microbial respiration rates off Mauritania and Senegal are high (**Chapter III**), suggesting the release of high amounts of bioavailable dissolved inorganic nutrients, which has the potential to stimulate regenerated phytoplankton growth. This is in line with previous studies, which have shown that despite the prominent upwelling off Cape Blanc, less than half of the primary production in the coastal zone off Mauritania and Senegal is actually "new". Remineralization of organic matter by heterotrophic bacteria supports the remainder (ca. 2 gC m^{-3} yr^{-1}) of the local primary productivity (Auger et al. 2016).

Overall this indicates that autotrophic and heterotrophic bacteria play an important role in the food web of the NW African upwelling system and might thereby even contribute to the high biomass of fish in the EBCs.

Ecosystem responses to eutrophication

Eutrophication has affected a large range of marine and limnic ecosystems (Conley et al. 2009; Smith and Schindler 2009). In many regions a so called "regime shift" has taken place, where continuous eutrophication stressed ecosystems beyond what they can endure, resulting in an abrupt change in status, such as fundamental changes in ecosystem processes (Conley et al. 2009). Especially coastal ecosystems are declining world-wide as a response to anthropogenic stress and eutrophication (Sanchez-Cabeza and Druffel 2009). However, their ecosystem services, such as carbon sequestration and biodiversity conservation, are highly valuable (Ahmed et al. 2017).

Intense aquacultures practices in coastal marine environments often represent one source of anthropogenic eutrophication. Fish feeding, fish fecal material and stimulation of phytoplankton blooms lead to high POM concentrations in the water column (Alongi et al. 2003; Sarà et al. 2004). We have shown that due to the direct impact of aquacultures on POM concentrations, composition and microbial colonization, particles may serve as indicator for the impact of aquaculture activities on adjacent coastal ecosystems (**Chapter IV**). This represents a great opportunity to evaluate aquaculture impact on coastal ecosystem services in the future.

However, in order to uncover the many different facets of eutrophication, it has previously been argued that eutrophication research needs to be approached more interdisciplinary to really understand ecosystem responses rather than just focusing on impacts on the primary producers (Duarte 2009).

ACUTE: AquaCUlture practice in Tropical coastal Ecosystems

In this context the project ACUTE can be seen as a prime example, where a biogeochemist (WP1), microbiologist (WP2), fish physiologist (WP3) and social scientist (WP4) have worked together in order to better understand aquaculture practices in the eutrophic waters off Bolinao. This interdisciplinary work has revealed that the environmental conditions in Bolinao are highly variable on long (seasonality) and short (weather, tidal cycle, tidal amplitude and diurnal cycles) time scales. Furthermore, the interdisciplinary research of ACUTE can suggest a feedback loop, how the rearing of milkfish and the eutrophic environmental conditions interact in the region:

Overfeeding of and secretion of feces by *Chanos chanos* result in aquaculture effluents, rich in inorganic nutrient and OM concentrations. This promotes the formation of large organic

particles, which in this area are mainly comprised of microalgae, sediment grains, fish feces and fish pellets. The overlap in microbial communities observed in the milkfish gut and on local macro-particles further indicates that fish feces are incorporated into particles. Those particles, which rapidly sink to the sediments below the fish cages, accumulate there and stimulate bacterial activity, often leading to hypoxic conditions. The overlap of microbial communities in sediments and milkfish gut highlights the role of sinking particles in promoting the vertical connectivity of the water column and the underlying sediments. The higher organic matter concentrations in water column and sediments in turn affect the reared fish again, closing the loop. Interestingly, the results of WP3 indicate that such high POM concentrations do not directly cause increased stress in milkfish. The robust species can tolerate high turbidities and other environmental fluctuations. Their stress in the waters off Bolinao was primarily determined by management strategies, such as stocking densities. However, indirectly high POM concentrations can affect the performance, welfare and even survival of milkfish, if high microbial respiration leads to hypoxia.

Hypoxia: Oversupply of organic matter and resulting microbial respirations can affect ecosystem health

While the economic loss resulting from fish kills in Bolinao can be in the range of millions of dollars, as it was the case in 2002 (San Diego-McGlone et al. 2008), the environmental consequences of hypoxia are also detrimental. The occurrence of hypoxia is already an alarming signal, indicating that ecosystem thresholds may be approached and management of nutrient and organic matter input is required. Thus, the understanding and conservation of sustainable environmental functioning in the Bolinao channel is pivotal for both the ecosystem and to prevent further losses in the local aquaculture industry.

Results of **Chapter III** indicate that high particle-attached microbial respiration rates may contribute to the low oxygen/ hypoxic conditions in the water column regularly observed in Bolinao.

Hypoxia is an especially problematic consequence of eutrophication as reoccurring oxygen depletions can alter ecosystem functions and even lead to a feedback loop of self-sustaining hypoxia (Conley et al. 2009): On top of its negative effects on living organisms, hypoxia also affects biogeochemical cycling of nutrients, since microbial activity in anoxic sediments often lead to the leakage of additional dissolved inorganic phosphorous (DIP) into the water column (Jensen et al. 1995). At the same time anoxia can reduce nitrogen loss by hindering microbial processes such as denitrification (Seitzinger and Giblin 1996). As a result, even more

dissolved inorganic nitrogen (DIN) and DIP is available for primary productivity, thereby possibly sustaining eutrophication and hypoxia (Conley et al. 2009). Elevated phosphorous levels have also been reported in Bolinao and have been attributed to hypoxia events (Ferrera et al. 2016).

All in all, this highlights the possible negative effects of microbial carbon degradation under excess POM conditions on coastal ecosystem functioning and health.

Recovery potential of Bolinao's coastal marine environment

Although the deterioration of the Bolinao coastal environment has progressed far, serious and consequently applied environmental conservation measurements could still promote its recovery. The most straight forward solution constitutes the reduction of fish farming structures in the channel. After a major fish kill in 2002 (Azanza et al. 2005; San Diego-McGlone et al. 2008) the numbers of fish farming structures were reduced by around half in the channel, thereby respecting its calculated carrying capacity (Ferrera et al. 2016). Only a few weeks after the sampling campaign for Chapter IV another fish kill (although apparently not due to hypoxia) took place in the area, after which the fish cage operators were prohibited to restock their empty cages for two months (Desrianti personal communication). These measures reduce the nutrient and organic matter input, which is an important step for reducing microbial carbon turnover. Personal communication with local fish farmers indicated that individual operators go even further in preventive measures. Some merely supply fish feed to the system when waters show low turbidities (e.g. during incoming tides). This indicates that progress has been made and the environmental awareness has risen in Bolinao. However, fishermen in the municipality of Anda, whose waters are adjacent to that of Bolinao, do not apply such countermeasures and instead, the number of fish-farming structures is constantly increasing. This may be one of the reasons why Bolinao waters do not seem to undergo remission, despite the continuous efforts. In consequence, both Anda and Bolinao need to work on common solutions for greening the aquaculture activities in the area.

Organic particles: micro-habitats for marine bacteria

Despite the major ecosystem differences between the two study sites of this thesis, the local marine POM always served as habitat for many marine bacteria. This is in line with numerous previous studies showing that certain groups of bacteria have the ability to attach to and thrive on particles (Crump et al. 1999; Hollibaugh et al. 2000; Ortega-Retuerta et al. 2013; Bižić-Ionescu et al. 2014; Rieck et al. 2015; Thiele et al. 2015; Datta et al. 2016; Zhang et al. 2016; Mestre et al. 2017; Pelve et al. 2017).

Why do some groups of bacteria attach to marine particles?

The life styles of marine bacteria

For a long time, it was assumed that marine bacteria are homogeneously distibuted in the water column. Nowadays, the awareness that micro-scale gradients and hotspots of nutrients and organic matter exist in the ocean is increasing (Grossart 2010; Stocker 2012). Motility and chemotaxis of marine bacteria allow them to exploit patches of high organic matter concentrations (Grossart et al. 2001; Stocker 2012).

While typical FL bacteria, such as SAR 11, are adapted to low nutrient conditions by possessing streamlined genomes and cell sizes (e.g. SAR 11), the high OM concentrations in particles have selected for efficient biopolymer degraders, such as Planktomycetes and Verrucomicrobia (Delong et al. 1993; Crump et al. 1999; Martinez-Garcia et al. 2012; Yung et al. 2016). Especially in oligotrophic environments the attachment to particles provides important services to bacteria including for example continuous supply of nutrients and OM. Therefore, these paricles are often referred to “microbial hotspots” in oligorophic oceans (Grossart 2010)

In eutrophic systems, background OM and nutrients are also high. Thus, the provision of a conventient food source may not be sufficient to explain why bacteria attach to organic substrates. Other theories have been proposed. These include for example refuges from predation, phages and abiotic stress and “buffering” of environmental fluctuations, making particles “micro-islands” for bacteria (Lyons et al. 2010; Tang et al. 2011; Yung et al. 2016).

Microscale dynamics on particles

The microscale dynamics on marine particles are very complex (Simon et al. 2002; Grossart et al. 2003; Kiørboe et al. 2003). The current understanding suggests that fresh marine particles are rapidly colonized by primary particle degraders, followed by the invasion of

secondary consumers (Datta et al. 2016). Additionally, a trade-off between colonization and dispersal of two populations of the same species of bacteria has been unraveled (Yawata et al. 2014), suggesting that some bacteria firmly attach to particles upon their arrival, while others loosely associate so that they can detach again if other hotspots are in close vicinity. The results of this thesis indicate that under natural environment these interactions are further complicated due to the influence of changing environmental conditions. Furthermore, the encounter of different water masses (and thus ambient BCC) during vertical and horizontal export of particles can affect microbial particle dynamics:

In this study (**Chapter II**) we have identified environmental parameters, such as temperature, salinity and substrate availability, which can promote the exchange (i.e. attachment/ detachment) between FL and PA bacteria. We suggest that higher particle concentrations in the environment lead to more frequent exchange between PA bacteria, which have to cross the FL state to reach the next particle. In our study this was the case at the deep chlorophyll maximum. However, also oligotrophic vs. eutrophic ecosystems could provide natural laboratories to study the similarities/ dissimilarities and thus exchange of FL and PA communities further.

During settling, particles encounter different water masses. This was simulated in **Chapter III**, where we observed exchange of the large (<0.3 mm) substrate-attached BCC and the BCC of the surrounding water masses. Chemotaxis and motility of suspended bacteria may allow them to approach particles and attach to them (Grossart et al. 2001; Stocker 2012). However, these particles are often surrounded by DOM plumes (Kiørboe and Jackson 2001), which may be difficult for FL bacteria to cross, especially if particles are large and fast sinking (Iversen, personal communication). Another possibility, which could connect the established particle-attached communities with the surrounding sea water BCC, is collision or scavenging. When larger, faster sinking particles collide with smaller particles, depending on their stickiness, they and the BCC can be incorporated into the larger particles. Future studies could investigate if there was a correlation between TEP concentrations (increasing stickiness) in sinking particles and their exchange with the surrounding water column/ smaller PA BCC.

Particles as vectors for pathogenic bacteria

Previous research in our working group “Tropical marine microbiology”at the ZMT, has suggested a stimulation of potentially pathogenic marine bacteria under eutrophic,

polysaccharide rich conditions (e.g. Kegler et al. 2017; Cárdenas et al. 2018). Especially, the effluents of fish and shrip farming structures can contain potentially pathogenic bacteria (Garren et al. 2009; Alfiansah et al. 2018). In the waters off Bolinao, an outbreak of *Vibrio cholera* occurred in 2005 (Reichardt et al. 2007, 2013). The prevailance of *V. cholera* in aquatic environments is facilitated by their ability to attach to various sorts of substrates (Hood and Winter 1997; Tarsi and Pruzzo 1999). Therefore, particles in the waters off Bolinao may also play an important role in desease ecology (Lyons et al. 2007).

CONCLUSION AND OUTLOOK

In this thesis the role of particles as vectors for carbon export, indicators of aquaculture impact and microbial hotspots, refuges and micro-islands was investigated in two very contrasting marine ecosystems. Off Mauritania and Senegal, FL and PA bacteria were described for the first time and potential key players for carbon turnover were identified. In the same study area, high carbon respiration rates indicate an important role of bacteria in determining the efficiency of carbon export to the deep ocean. Future studies in EBC systems should focus on PA/ AG BCC and their activities as this may help further elucidate the effect of bacterial activity on the BCP efficiency. Especially a more detailed estimate about the contribution of specific microbial groups (e.g. Planktomycetes and Verrucomicrobia) on the degradation of organic matter can aid in linking abundances and functionality better.

The results of this thesis indicate that environmental parameters, such as temperature, salinity and substrate availability play important roles in microbial particle dynamics. Results suggest that if substrate availability was high (e.g. at the deep chlorophyll maximum), PA bacteria switched between hotspots, thereby rendering FL and PA fractions more similar. Since eutrophic coastal environments are also characterized by high substrate availability, it would be interesting to systematically compare the effect of eutrophication on the similarity/ dissimilarity of FL and PA bacteria in the future.

Exchange between substrate attached bacteria and the surrounding sea water BCC was also observed in sinking aggregates. Here, scavenging of smaller particles may be responsible for the observed changes. To investigate this hypothesis further, future studies may combine microbial community analyses with measurements on the stickiness (i.e. TEP content) of sinking aggregates.

Results from the aquaculture impacted coastal region off Bolinao indicate that particle characteristics (abundances, CN ratio, stable isotope values, bacterial alpha diversity and BCC) can serve as indicators for the impact of aquaculture activities on adjacent ecosystems. Furthermore, high organic matter concentrations resulting from aquaculture activities led to high microbial respiration rates, which have the potential to contribute to recurring hypoxic conditions in the water column off Bolinao.

ACUTE has provided compelling evidence on the environmental impact of aquaculture activities off Bolinao. In order to improve environmental conditions in the area, future

research should focus on the effect of nutrient and organic matter input in the adjacent municipality of Anda, too. Since Bolinao and Anda share the same water body, connected by tidal movements, they need to work on a common action plan on how to reduce the environmental impact of their shared main economic activity. The negative feedbacks, occurring in the coastal waters off Bolinao, suggest that this should be done sooner rather than later to prevent any further environmental degradation.

Taken together the results of this thesis provide evidence that heterotrophic processes occurring at the microscale on marine particles may have the ability to influence global sequestration of organic carbon and coastal ecosystem functioning.

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CHAPTER VI

APPENDIX

Evaluating impacts of intensive milkfish aquaculture on water quality, organic matter loading, and bacterial communities in Bolinao, Philippines

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ABSTRACT

Intensive milkfish aquaculture is gaining in importance to provide fish for local consumption in the Philippines, irrespective of ecological and health concerns. To promote more sustainable aquaculture practices, this study investigated the effects of intensive open-cage aquaculture on local water quality, organic matter (OM) loading, and bacterial communities. We sampled the water column at five stations along the Guiguiwanen Channel (Bolinao, Philippines), covering a gradient from the entrance of the channel, without fish cages and a more pronounced influence of the open ocean, to the secluded interior, exploited by numerous fish cages. Intensive aquaculture led to a reduced pH (7.8), oxygen concentrations close to hypoxia, and two to five times elevated concentrations of inorganic nutrients. Tidal currents moved water with increased particulate OM loading from the interior of the channel to areas otherwise less affected by aquaculture. Carbon to nitrogen ratios of these particles suggested that they derived primarily from fish feed (C:N ratio 8). Full-length amplicon sequencing of the 16S rRNA gene will further provide data on bacterial communities in the water column (both free-living and particle-attached) at a high taxonomic resolution. Thereby, we aim to identify potentially harmful strains, supplementing the sequence-based analysis by quantitative PCR of marker genes for pathogenic bacteria, such as *Vibrio parahaemolyticus* and *V. cholera*, and antimicrobial resistance genes. Our goal is to provide a holistic assessment of the effects of intensive open-cage aquaculture, identifying potential indicators for detrimental impacts on animal and human health for more sustainable management of this rapidly growing industry.

Keywords: tropical marine aquaculture, sustainability, water quality, organic matter input, pathogenic bacteria, 16S sequencing

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